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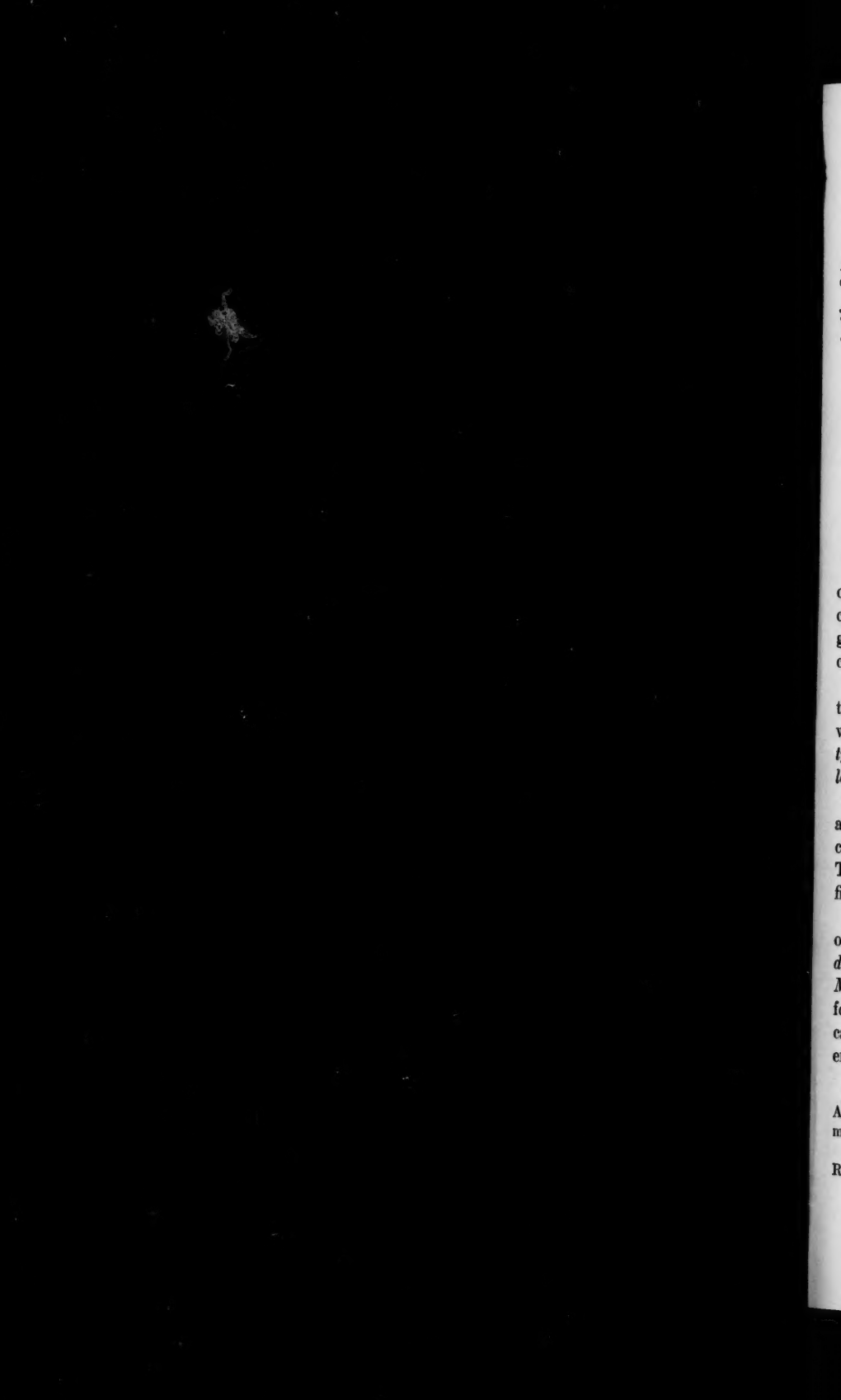

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Pathogenicity of Five Isolates of Root-Knot Nematodes (*Meloidogyne* Spp.) to Sugarcane Roots[1]

Jessé Román[2]

INTRODUCTION

The agricultural economy of the Island of Puerto Rico is mainly based on the production of sugarcane. A major part of the land available for cultivation has been devoted to this purpose for many years. Although a great deal of research has been conducted on the diseases and pests of this crop, the possible effects of nematodes have been considered very little.

During investigations carried out in Puerto Rico in 1957–58, to determine the types of parasitic nematodes associated with sugarcane roots, species within the following genera were found: *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, *Hoplolaimus*, *Criconemoides*, *Xiphinema*, *Longidorus*, and *Ditylenchus*.

There are plans to conduct a series of investigations to determine whether any of these genera of plant-parasitic nematodes are detrimental in the cultivation of this crop. The present work is a part of this long-range project. The root-knot nematode genus, *Meloidogyne*, was selected for consideration first, because it is most frequently present in soil and root samples.

To date only three root-knot nematode species have been identified as occurring in the roots of sugarcane in the Island. These species are *Meloidogyne incognita incognita*, *M. incognita acrita*, and *M. arenaria*. Of these, *M. incognita acrita* seems to be the most widely distributed. It has been found that races or strains exist within this species which are morphologically indistinguishable, but which can be separated by host-range differences.

[1] This work was the basis for a thesis submitted to the Graduate Faculty of the Alabama Polytechnic Institute, Auburn, Ala., in partial fulfillment of the requirements for the degree of Master of Science.

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Although root-knot nematodes penetrate, form galls, and multiply in sugarcane roots, it has been reported (*1*, *5*)[3] that they do little or no damage to this crop. In contrast to this, other authors (*2*, *14*) have reported that these nematodes may be detrimental. This work was performed in an effort to determine objectively what damage, if any, this nematode does to sugarcane roots.

Two different but related lines of investigation were conducted, the first as to the nature of the pathogenic effects of the nematodes; and the second concerning the suitability of sugarcane as a host for root-knot nematodes. Special efforts were also made to determine whether *M. incognita incognita*, *M. incognita acrita*, and *M. arenaria* exhibit differences in pathogenicity to sugarcane roots, and to assess the possibility of the existence of strains within *M. incognita acrita*.

Several commercial varieties of sugarcane are grown in Puerto Rico, but, to limit the scope of this investigation, only one variety was used. A recently produced variety, Puerto Rico 980, was selected since it produces the highest yield of sugar per acre per month. It is also highly resistant to some of the major diseases, and it is expected that this variety will be widely used.

LITERATURE REVIEW

Treub (*21*) in 1885, reported that *Heterodera javanica* attacked sugarcane roots in Java. This is probably the earliest report of root-knot nematode infection in sugarcane. He said that the larvae entered through the root tip and also may have penetrated the root through breaks or openings in the root surface. He found that the larvae move intercellularly to the primordium of a lateral root to start their feeding. He also noticed that five or six giant cells formed around the region of the nematode's head. These cells have thick walls and 30 to 40 nuclei each. Treub reported that both the central cylinder and the cortex of the root were involved in the formation of a gall.

Matz (*14*) in 1925, on the other hand, reported that galls in sugarcane roots were enlargements of the outer fleshy layer of the root only, while the central part was not deformed. In the same paper, he reported results of field studies with sugarcane infected by root-knot nematodes on the south coast of Puerto Rico. He described the above-ground symptoms as intense, waxy, golden-yellow colored bands in the leaves starting from the tips and extending to the sheaths in older plants. In young plants, 3 to 4 months old, the leaves became shriveled and streaked longitudinally with red, copper-colored areas divided by long dry areas. He claimed that heavily infected plants were characterized by a marked stunting, and that galls,

[3] Italic numbers in parentheses refer to Literature Cited, pp. 83–4.

which occurred mostly at the root tips, were about twice the diameter of the unaffected root in size. Boyd (*2*), working on sugarcane in Georgia in 1925, reported that this marked stunting could be used to detect root-knot nematode infestations in sugarcane fields. He also observed that the size of the gall attained diameters up to one-quarter of an inch.

While these investigators emphasized the importance of root-knot in sugarcane, Cook (*5*) in 1925, stated that this disease was of little importance in Puerto Rico. In addition to this Muir (*16*) in 1926, Zwaluwenburg (*22*) in 1929, and Jensen, *et al.* (*9*) in 1959, reported that root-knot of sugarcane in Hawaii was not very important, because gall formation did not lead to a breakdown of the roots. Some investigators (*9*, *14*, *22*) also emphasized that excessive numbers of lateral roots were formed around the galled region.

Cavities or pits, about one-half millimeter in diameter, are frequently found on sugarcane roots. These pits are formed in the cortical tissue and, generally, the central cylinder is not affected. This condition has been noted by investigators for many years. Edgerton and Tims (*7*) in 1927, investigating the sugarcane-disease situation in Louisiana, considered pitting to be caused by soil animals such as snails, centipedes, springtails, and nematodes. However, Treub (*21*), as early as 1885, reported that the cavities he studied were associated with root-knot nematodes because there were giant cells in their vicinity. Evidently, he is the only worker to have published such an observation. Matz (*14*) also came to the conclusion that cavities were caused by nematodes, but did not mention the giant cells reported by Treub.

A few other investigators published interesting statements regarding sugarcane attacked by root-knot nematodes. Bessey (*1*) in 1911, found that nematodes were abundant in root-knot-infected sugarcane roots, but their injury to the roots apparently was not great. Spencer (*19*) in 1919, found that some of the most important sugarcane-growing sections of Florida were "seriously infected" with root-knot nematodes. Martin, *et al.* (*13*) in 1956, found *M. incognita acrita* in sugarcane in Louisiana. Details regarding symptoms and pathogenicity were not mentioned by any of these workers.

Other than the previously mentioned work by Treub, there apparently is no published information regarding the cytology of sugarcane roots attacked by root-knot nematodes. The literature review which follows, although based on other crops, is pertinent to the cytological studies performed by the author.

Kostoff and Kendal (*11*) in 1930, studying the cytology of root-knot nematode galls on tobacco roots, found that giant cells were formed by an abnormal type of mitotic division. In this division cell walls did not form between the separated chromosomes; this resulted in multinucleate cells

the nuclei of which appeared in multiples of two. These nuclei agglutinated, their nuclear membranes dissolved, and their contents coalesced.

Christie (*4*) in 1936, studying root-knot nematode galls on tomato roots, concluded that entering larvae migrated intercellularly and finally located with their heads in the plerome near the beginning of the region of cell elongation. In contrast to the reports of Kostoff and Kendal (*11*), Christie observed that cell walls of cells receiving stimulation by the nematode dissolved, and their cytoplasmic contents coalesced to begin the formation of a giant cell. He agreed with the results of Kostoff and Kendal that the nuclear membranes dissolved and nuclei coalesced. He further pointed out that the pericycle was stimulated by the nematode, and this resulted in lateral root formation.

Krusberg and Nielsen (*12*) in 1957, found the intercellular migration of larvae, as reported by Christie (*4*) to occur in tomato roots, and also in the roots of sweetpotato, but only through the cortical tissue. Migration through the vascular tissue was intracellular. The dispersed nuclei of an individual giant cell acquired a pyriform shape, aggregated together with their narrow ends towards a central point, and then coalesced. These authors (*12*) also reported what appeared to be an abnormal condition of the xylem. This abnormality apparently was formed directly from xylem parenchyma and was characterized by secondary wall thickenings of annular, reticulate, or pitted elements. Although commonly associated with giant cells, this abnormality was considered to be a response of the xylem to injury. Krusberg and Nielsen considered that this injury, perhaps, is not peculiar to root-knot nematodes since it was also noted in association with necrotic cells in traces of sloughed-off lateral roots.

MATERIALS AND METHODS

SOURCES OF NEMATODES AND PREPARATION OF CULTURES

Five isolates of root-knot nematodes were collected in 1958 from various host plants in Puerto Rico and identified as follows: *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949; *Meloidogyne incognita incognita* (Kofoid and White, 1919) Chitwood, 1949; and three selections of *Meloidogyne incognita acrita* Chitwood, 1949, from three different hosts.

M. arenaria was collected from roots of pepper (*Capsicum annuum* L.) from a field at Río Piedras used for growing vegetables the previous 5 years. *M. incognita incognita* was collected from tobacco (*Nicotiana tabacum* L.) roots from a field at Isabela which had been in tobacco the previous 3 years. One selection of *M. incognita acrita* came from the roots of pineapple (*Ananas comosus* Merrill) in a field at Manatí where pineapples had been grown for the previous 7 years following sugarcane. The second

selection of *M. incognita acrita* was taken from the roots of cucumber (*Cucumis sativus* L.) grown at Corozal where vegetables had been growing for 1 year. The field had been used for sugarcane for a number of years prior to this. The third selection of *M. incognita acrita* was obtained from the roots of "culantro del monte" (*Eryngium foetidum* L.) which, along with weeds, had been growing for 5 years in a field located at Río Piedras.

Cultures of the nematode isolates were initiated from single egg masses taken from a female of each selection possessing a typical perineal pattern for the nematode species desired. Each of the five selected egg masses was introduced into the root zone of a separate tomato seedling grown in steam-sterilized soil. After about 90 days, egg masses were removed from the 5 plants on which the nematodes had been cultured and were transferred to 20 additional tomato plants for the further increase of the nematode isolates. Each group of isolates was kept on an individual bench to avoid cross-contamination.

TREATMENTS

In preparation for the treatments, sugarcane cuttings of the variety Puerto Rico 980 (P.R. 980) were grown in 10-inch clay pots in steam-sterilized soil. These plants, when 8 to 10 inches high, were transferred to halved steel drums of 55-gallon capacity filled with steam-sterilized soil. The drums had holes drilled in the bottoms for drainage and were raised from the ground by cement blocks.

Inoculum for the treatments was then obtained from the tomato plants in which the nematodes were cultured. Egg masses were removed from the roots and placed in water, and, after most of the eggs had hatched, approximately 5,000 larvae were counted and placed in a beaker containing 75 ml. of water. After 4 hours, the top 50 ml. were decanted through a 325-mesh sieve that retained all nematodes but permitted passage of other micro-organisms which might be present. These nematode-free supernatant water collections were used as treatments to determine the effects, if any, of these micro-organisms. The remaining suspensions in the beakers containing the nematodes plus other micro-organisms were used as separate treatments. Inoculum was applied to the sugarcane plants in the steel drums by pouring the nematode suspension or supernatant water through a funnel inserted in the soil at the base of the plants.

A total of 44 sugarcane plants in individual drums was used. Four drums, each containing one sugarcane plant, were inoculated for each nematode and for each supernatant treatment from the five different nematode isolates. Plants in four drums were left uninoculated to serve as controls.

The treated plants were allowed to grow 5 months before being harvested for examination. No fertilization was used in this short growing period.

Routine leaf-insect control was accomplished by the use of Dieldrin. Cardboard shields placed over the drums prevented accumulation of insecticide in the soil.

PREPARATION OF ROOTS FOR EXAMINATION

The sugarcane plants were removed from the soil and their roots washed thoroughly. Individual quantities of 200 gm. of roots were obtained by taking small bunches of roots from different parts of each plant. These roots were fixed in formalin-aceto-alcohol following the method of Sass (*17*) before being transported from Puerto Rico to Auburn, Ala., where the laboratory phases of the work were done.

For histological studies, root-apex pieces about 1½ cm. in length were selected from roots of three categories. These were primary roots which developed directly from the shoot, secondary roots which developed from the primary roots, and tertiary roots which developed from the secondary roots. All root pieces were dehydrated, infiltrated, and embedded in paraffin according to the method of Johansen (*10*). Longitudinal and transverse serial sections were cut 12 μ in thickness with a rotary microtome and triple stained with safranin, fast green, and orange G combination. These sections were examined for details of larval penetration, migration, and other pathological effects on the tissues and cells. The nature of the cortical cavities or pits was also investigated.

Gross effects of the treatments on the sugarcane roots were studied by placing the FAA-fixed root samples in water trays. Observations on root distortions, coloration, cavities, lesions, and galling were made with the aid of a hand lens.

Quantitative studies were made of the roots as to the nematodes within, root galls, lateral roots, and cortical cavities. For this purpose samples of the FAA-fixed roots were taken for each treatment and divided into the primary-, secondary-, and tertiary-root categories. In all cases pieces of roots containing the root tips were used and stained with the simplified lacto phenol-acid fuchsin method of McBeth, Taylor, and Smith (*15*) for staining nematodes *in situ*.

For each treatment 10 primary roots, 33 secondary roots, and 48 tertiary roots were examined under a dissecting microscope. Counts were made of the numbers of the nematodes in various recognizable stages of their life history, root galls, lateral roots exposed at the surface, and the cortical cavities or pits. These data were recorded for the first centimeter measured from the root apex, the next 1 to 3 cm., 3 to 5 cm., and 5 to 7 cm. back from the apex. This was possible for the long primary roots, but secondary roots rarely exceeded 5 cm. in length and tertiary roots were only about 3 cm. long.

RESULTS

DETERMINATION OF THE GROSS AND QUANTITATIVE EFFECTS OF ROOT-KNOT NEMATODES ON SUGARCANE ROOTS

Tables 1–6 contain the detailed quantitative results. The most important of these data are summarized in table 7. In this table only data from the

TABLE 1.—*Results of the inoculation test with* **Meloidogyne arenaria** *from* **Capsicum annuum**

Root category	Root zone (cm.)	Infective larvae	Older larvae	Young females	Mature females	Males *in situ*	Root galls	Lateral roots	Root cavities
Primary	0–1	70	63	62	23	0	7	62	2
	1–3	28	13	71	28	0	3	115	8
	3–5	35	0	38	18	0	2	106	5
	5–7	13	0	12	12	0	0	37	4
Secondary	0–1	21	19	22	2	1	3	74	2
	1–3	12	8	28	5	0	1	101	4
	3–5	3	1	4	0	0	0	38	3
Tertiary	0–1	1	24	7	1	0	3	—	1
	1–3	7	7	3	0	0	1	—	0

TABLE 2.—*Results of the inoculation test with* **Meloidogyne incognita incognita** *from* **Nicotiana tabacum**

Root category	Root zone (cm.)	Infective larvae	Older larvae	Young females	Mature females	Males *in situ*	Root galls	Lateral roots	Root cavities
Primary	0–1	63	104	84	3	1	8	20	9
	1–3	27	31	216	25	9	12	94	13
	3–5	10	6	84	17	2	4	88	10
	5–7	25	2	41	11	4	2	50	7
Secondary	0–1	79	118	104	7	4	29	77	6
	1–3	5	5	26	0	0	5	161	10
	3–5	1	0	13	0	0	1	9	1
Tertiary	0–1	36	88	54	3	3	10	—	0
	1–3	18	20	43	6	4	3	—	0

first 3 cm. of the root tips are presented and these represent totals for all three root categories.

Roots from the plants inoculated with supernatant water were free from galls and nematodes (table 7), and, in appearance, were very similar to the

TABLE 3.—*Results of the inoculation test with* **Meloidogyne incognita acrita** *from* **Ananas comosus**

Root category	Root zone (cm.)	Infective larvae	Older larvae	Young females	Mature females	Males *in situ*	Root galls	Lateral roots	Root cavities
Primary	0–1	27	3	1	0	0	1	25	6
	1–3	3	2	17	1	1	2	34	8
	3–5	2	1	9	2	0	1	37	7
	5–7	3	0	0	0	0	0	36	9
Secondary	0–1	8	11	5	0	0	2	52	2
	1–3	4	5	3	0	0	1	101	3
	3–5	1	7	0	0	0	1	38	1
Tertiary	0–1	4	5	2	0	0	2	—	0
	1–3	2	2	1	0	0	1	—	0

TABLE 4.—*Results of the inoculation test with* **Meloidogyne incognita acrita** *from* **Eryngium foetidum**

Root category	Root zone (cm.)	Infective larvae	Older larvae	Young females	Mature females	Males *in situ*	Root galls	Lateral roots	Root cavities
Primary	0–1	19	89	71	0	0	8	34	7
	1–3	7	20	105	38	2	8	81	6
	3–5	4	5	114	82	2	6	92	7
	5–7	7	0	94	69	0	2	61	4
Secondary	0–1	17	76	48	0	0	8	35	3
	1–3	16	40	36	0	0	4	151	6
	3–5	0	3	0	0	0	0	24	1
Tertiary	0–1	18	59	43	0	3	16	—	2
	1–3	6	17	20	1	0	6	—	0

TABLE 5.—*Results of the inoculation test with* **Meloidogyne incognita acrita** *from* **Cucumis sativus**

Root category	Root zone (cm.)	Infective larvae	Older larvae	Young females	Mature females	Males *in situ*	Root galls	Lateral roots	Root cavities
Primary	0–1	43	104	172	107	9	9	49	14
	1–3	49	46	109	125	5	5	150	9
	3–5	29	0	61	55	6	4	107	8
	5–7	37	0	26	34	0	3	78	11
Secondary	0–1	45	82	55	3	2	16	112	7
	1–3	8	16	24	0	1	8	130	6
	3–5	4	5	17	1	0	3	46	2
Tertiary	0–1	26	82	14	1	2	17	—	4
	1–3	2	18	3	0	0	0	—	0

TABLE 6.—*Results of the inoculation tests with supernatant water from the 5 root-knot nematode cultures and the noninoculated control*

Root category	Root zone (cm.)	Results when inoculated with supernatant water from root-knot nematodes										Noninoculated	
		M. arenaria from pepper		*M. incognita*, from tobacco		*M. incognita acrita*							
						From pineapple		From *E. foetidum*		From cucumber			
		Lateral roots	Root cavities	Lateral roots	Root cavities	Lateral roots	Root cavities	Lateral roots	Root cavities	Lateral roots	Root cavities	Lateral roots	Root cavities
Primary	0–1	19	4	45	4	28	1	30	3	35	1	31	1
	1–3	75	4	96	8	70	1	99	4	64	2	94	3
	3–5	131	7	108	5	101	2	89	2	73	3	120	4
	5–7	120	5	100	2	87	2	54	1	38	1	86	2
Secondary	0–1	126	0	144	2	40	3	104	0	75	2	57	1
	1–3	147	3	187	1	106	2	206	2	168	5	132	3
	3–5	51	0	94	1	161	1	46	2	72	0	126	2
Tertiary	0–1	—	0	—	0	—	0	—	0	—	0	—	0
	1–3	—	0	—	0	—	0	—	0	—	0	—	0

roots from the noninoculated control plants. Roots under both these treatments were straight and exhibited only the normal, slight, wavy appearance. Their tips were normally tapered. Root color varied from white in the young roots, to brown and black in the old ones. Brownish-black lesions and cavities similar to those noted in the roots infected with nematodes were also observed, but the frequency with which they occurred was slightly lower in the nonnematode-infected roots.

The roots of the plants that were inoculated with the five root-knot nematode isolates had distinct curvatures. In all these treatments, curva-

TABLE 7.—*Summary of the quantitative data obtained for the first 3 cm. of all the roots under different treatments*

Treatment	Nematodes	Galls	Cavities	Lateral roots
Meloidogyne arenaria	526	18	17	352
M. incognita incognita	1,186	67	38	352
M. incognita acrita from pineapple	106	9	19	212
M. incognita acrita from E. foetidum	751	50	24	301
M. incognita acrita from cucumber	1,153	55	40	441
Control	0	0	8	314
Supernatant water of *M. arenaria*	0	0	11	367
Supernatant water of *M. incognita incognita*	0	0	15	372
Supernatant water of *M. incognita acrita* from pineapple	0	0	7	244
Supernatant water of *M. incognita acrita* from *Eryngium foetidum*	0	0	9	439
Supernatant water of *M. incognita acrita* from cucumber	0	0	10	342

tures were most pronounced in the most highly galled roots. Root curvature occurred preceding a gall, after a gall, or in between galls (fig. 1).

The color of the roots under all nematode treatments ranged from white in the young roots, to brown and black in the old ones. No differences in root coloration were found between treatments.

Superficial, brownish-black lesions were observed scattered over the entire length of roots inoculated with each of the five nematode isolates. These lesions ranged from 1 to 3 mm. in length, and varied from elongated to irregular in shape (fig. 2). The older roots were so dark in color that the extent of their lesions could not be determined.

In contrast to these shallow lesions, cavities were found in roots of all ages extending through the cortex as far as the central stele. These cavities, as seen at the surface of the root cortex, appeared as tiny holes about ½ mm. in diameter (fig. 3). No external damage was seen in the tissue sur-

rounding the apertures of these cavities. No differences were observed regarding the superficial appearance of the cavities occurring in the roots

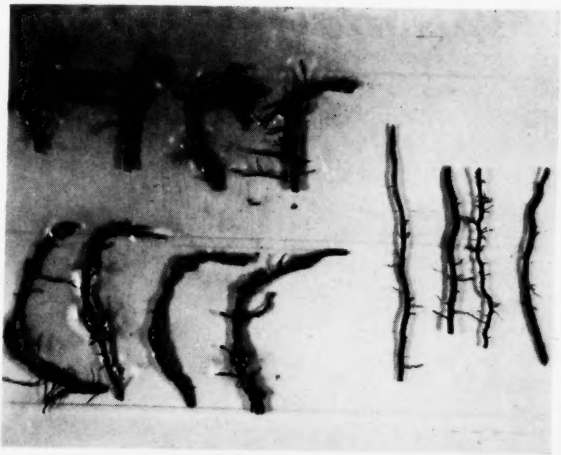


FIG. 1.—Contrast between infected curved and straight control roots of sugarcane.

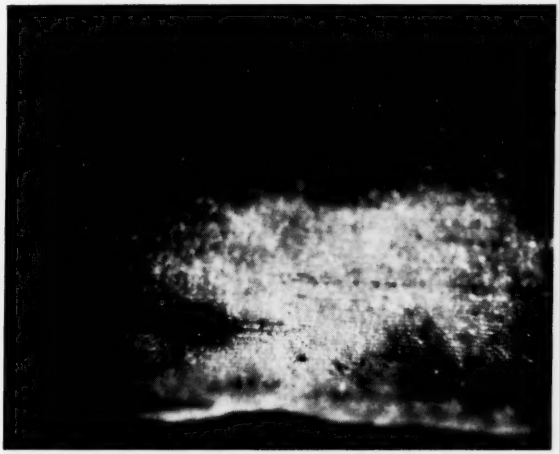


FIG. 2.—Superficial lesions present in sugarcane roots.

exposed to each of the root-knot nematodes and supernatant treatments. No differences in appearance of the cavities were found between the treated and uninoculated control roots. However, the numbers of cavities and galls

were found to be higher when the number of nematodes within the first 3 cm. of root tips was higher (table 7).

Galls in the primary roots were higher in number per centimeter of root within the first 3 cm. from the apexes than at locations farther back (tables 1–5). Gall shapes were similar in all the five sets of nematode-inoculated roots, being spheroidal or ellipsoidal. Both occurred at the proximal as well as at the distal parts of the roots (fig. 4).

The total number of lateral roots was higher in the nematode and supernatant-water treatments than for the uninoculated controls (table 7), with

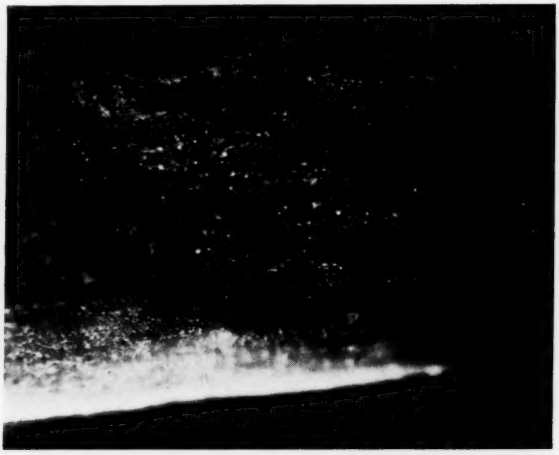


FIG. 3.—Surface view of cortical cavities.

the exception of those roots inoculated with *M. incognita acrita* from pineapple and its corresponding supernatant treatment.

In general, it was noted that, in each root category, the highest number of nematodes per centimeter of root usually occurred in the first centimeter from the root apex (tables 1–5). These nematodes represented all stages of development. However, the total number of males was found to be so low that no further study of them was conducted.

HISTOLOGICAL STUDIES

In the absence of root-knot nematodes, roots inoculated with the supernatant water and the noninoculated control roots gave no pathological responses. There were no differences observed in the internal responses of the roots infected with each of the five root-knot nematode isolates. Thus, the observations to be described in detail as to nematode penetration and

migration, sites for feeding and maturation, pathogenic effects, and root cavities, were found to apply equally to all five isolates.

Penetration and Migration

Penetration of root-knot nematode larvae into the roots of sugarcane appeared to occur only at the region of the root tip extending from root cap into the zone of differentiation. Both the infective larvae and the galleries or burrows made by them during penetration and migration were observed. The numbers of the infective larval stage ranged as high as 15 in the meristem of a single root. Infective larvae and galleries were less numerous in

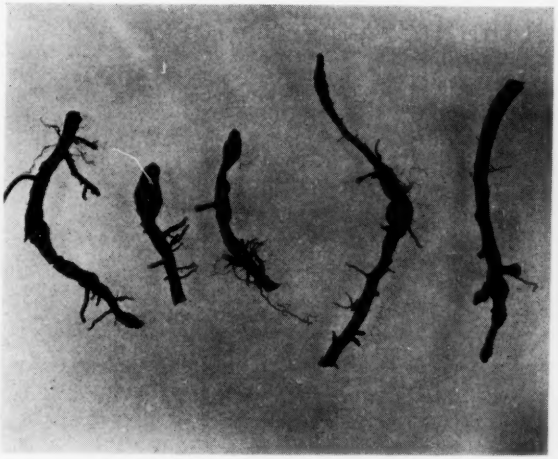


FIG. 4.—Large galls exhibited by root-knot nematode-infected sugarcane roots.

the root cap. In addition to penetration through the intact root cap, an instance was found in which a gallery left by a larva of *M. incognita incognita* in a secondary root connected with a break in the root cap (fig. 5).

No surface cell-damage was observed other than the entrance burrows made by the larvae. Direction of larval penetration in the four root-tip zones was found to be at different angles ranging up to about 90° with regard to the central axis of the root, but always oriented against the direction of root growth. Larvae were never seen oriented in the direction of root growth.

The galleries or burrows left by the infective larvae were composed of separated but intact cells as well as broken and empty cells. In these galleries there was no evidence of bacteria or fungi being present nor of damage caused by these organisms. It may be that the staining procedure used is

not suitable for revealing these other organisms. No discolorations or differences in staining were found in the tissues involved, nor was cell enlargement observed along these burrows (fig. 5).

These galleries were about the width of an infective larva and were nearly straight. In all cases only one larva was found to be associated with each burrow. In some instances portions of the burrows were found to be closed by unbroken cells. These cells may have been cells between which the nematodes had passed or, perhaps, they were altered in position by the process of embedding and sectioning.

Infective larvae and other stages were found within the vascular tissue.

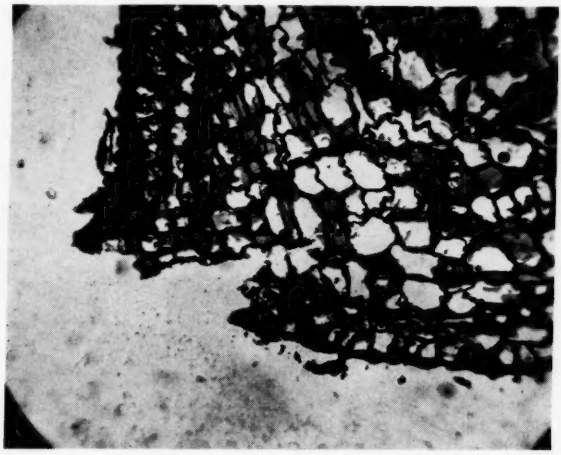


FIG. 5.—Gallery of a larva connected to a break in a sugarcane root cap.

However, since there was no evidence of a migration gallery through tissues of the vascular cylinder, it is possible that the nematodes had settled in this place before it became differentiated. Nematodes within the vascular cylinder, though scattered, were always oriented parallel to the walls of the cylinder with their heads directed away from the root tip.

Sites for Feeding and Maturation

The formation of giant cells and the production of egg masses were never observed in the root cap and meristematic zone. However, these occurred frequently in the zone of cell elongation. Egg masses and giant cells were observed in the older root tissues associated with nematodes lying entirely within the vascular cylinder or having their anterior portion inserted into the vascular tissue with the remainder of their bodies being in the cortex.

No females were found in the older, differentiated parts of the roots except in close proximity with the vascular cylinder. Only one instance was observed in which a female of *M. incognita incognita* in the cortical tissue did not have its head inside the vascular cylinder but did have giant cells around its anterior end (fig. 6).

Pathogenic Effects

The production of giant cells was the most important pathogenic effect caused by root-knot nematodes on the tissues of sugarcane roots. In order to get an understanding of the abnormal condition in the giant-cell area,

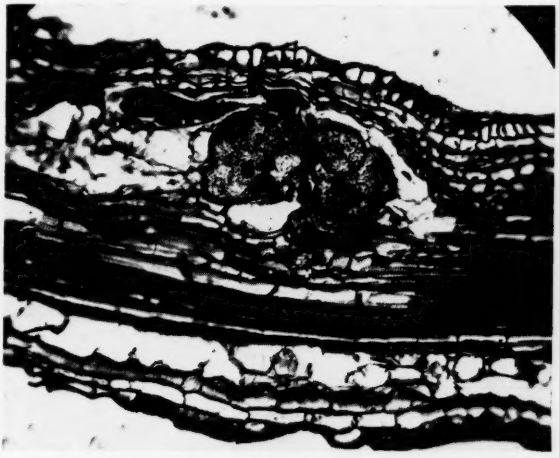
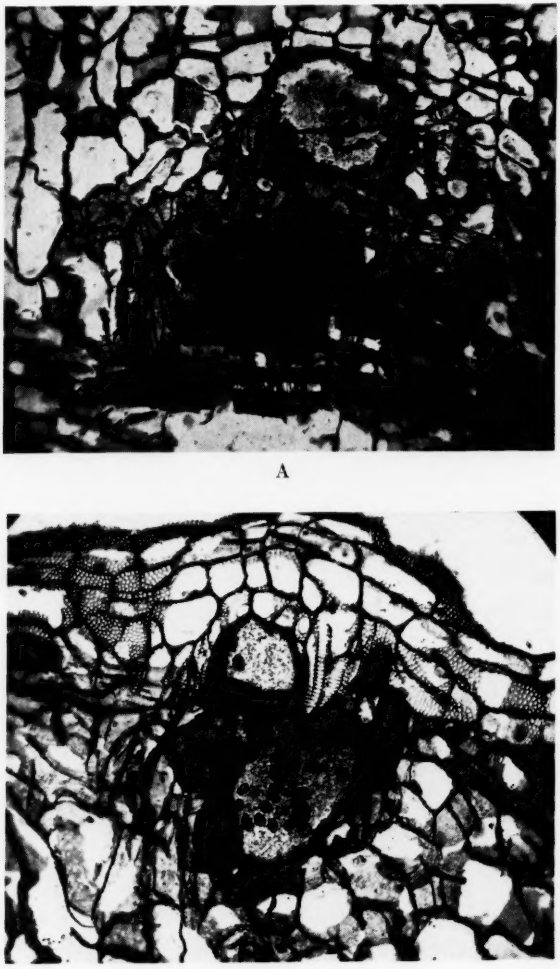


FIG. 6.—Female nematode with head not inserted into vascular tissue.

cells were studied first at the outermost part of the area and from there into the center where the nematode's head was located. The outermost cells had increased slightly in size, their cytoplasm and cell walls stained green as with all other unaffected cells, and their nuclei were very slightly enlarged and stained light red. Sometimes next to these cells, but closer to the center of the giant-cell area, a distinct hyperplastic zone was observed where the cells were numerous, small, and tightly packed together. Some of these had that part of their cell wall which adjoined the giant cells completely missing and represented cells in the process of coalescence. Mitotic figures were not observed in the giant cells although the staining technique did reveal them in the meristematic zones. The cells of the hyperplastic zone had walls and cytoplasm which stained the normal green with nuclei which were slightly enlarged but of the normal light-red color. There were a few cases

in which this hyperplastic zone was not detected even within the same root, although hyperplasia was generally present aroung the giant cells associated with mature females (fig. 7A).

Inside the giant-cell area, near the nematode's head, the cytoplasm of the cells became granular and stained light red. Cell walls stained green but



A

B

FIG. 7.— A, Hyperplasia around giant cells; B, fusion of giant-cell nuclei.

the cells and their nuclei were very much enlarged. These giant cells possessed many nuclei, each with conspicuous nucleoli which stained dark red. Groups of these nuclei fused together within the cells (fig. 7B).

In some of the cells around the nematode's head there was another type of nuclear fusion which perhaps followed the first fusion. Here the individual or previously fused groups of nuclei became flask-shaped and grouped together with their narrow ends pointing toward a central point (fig. 8A). This seemed to be followed by dissolution of the nuclear membranes and coalescence of their contents, resulting in a single amorphous mass of nucleoplasm (fig. 8B).

Cell-wall dissolution was very often seen in the giant cells (fig. 9A). This dissolution made possible the coalescence of cytoplasmic contents and resulted in the multinucleated condition of the giant cells. It is possible that the cell wall of the newly formed giant cells is composed of the undissolved portions from the other cells which joined in the coalescence.

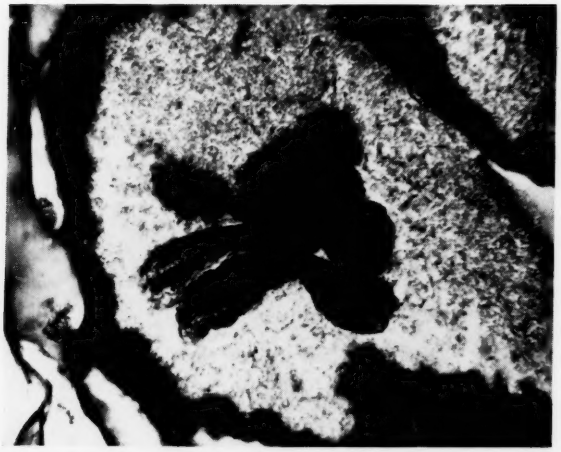
The shape of the giant cell varied with respect to the tissue from which it was formed. Giant cells formed from cells which would give rise to pericycle, endodermis, or cortical tissue were found to be generally rounded or oval. Those formed from the tissue which would give rise to vascular tissue were generally elongated (fig. 9B). Some giant cells close to the vascular tissue had internal knoblike structures which were part of the internal lining of the cell and were perpendicular to it. These were small and stained the same green color as the cell wall (fig. 10).

The sites for feeding and maturation already mentioned (region of cell elongation, cortical tissue with nematode's head inside vascular tissue, and vascular cylinder) were the places where the pathogenic effects were most pronounced.

Nematodes feeding in the region of cell elongation caused differentiation of most of this zone into giant cells. Large numbers of root-knot nematodes feeding at the beginning of this zone caused severe damage. Generally, a large terminal gall was found in which the characteristic small and closely packed meristematic cells of the apical meristem had partly disappeared and was replaced by large nonmeristematic cells. This condition was accompanied by the absence of a root cap and the presence of a few vascular vessels which were so close to the root apex that the region of cell elongation appeared considerably reduced (fig. 11A).

Nematodes with their anterior portions embedded into the vascular cylinder and their remaining portions lying in the cortex, and nematodes completely inside the vascular cylinder were observed also to have a giant-cell area around their heads. In both cases vascular vessels frequently curved around the giant-cell area (fig. 9B).

An abnormality of the xylem was found associated with nematodes feed-



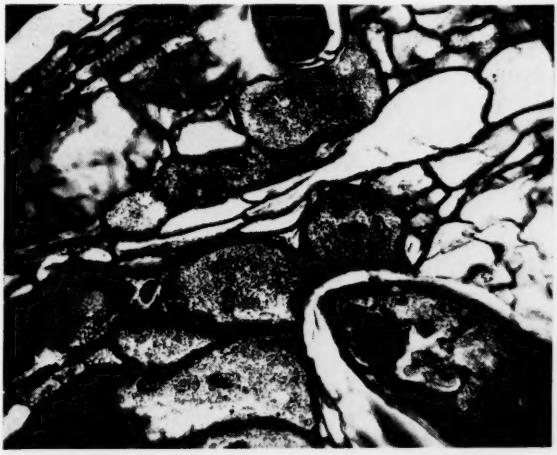
A

B

FIG. 8.—A, Fusion of flask-shaped nuclei; B, amorphous mass of nucleoplasm that appeared to result from fusion of the flask-shaped nuclei.

ing within the vascular cylinder. This abnormality which was characterized by the presence of xylem vessels disorderly arranged was found in the vicinity of the giant-cell area (fig. 11B).

The root-knot nematode not only caused damage to the plant tissue



A

B

FIG. 9.—A, Cell-wall dissolution between giant cells; B, elongated giant cells within the vascular tissue and curving of the vascular vessels.

surrounding the head of the nematode, but also damaged cells which surrounded the rest of the body. Since the nematode's body increases greatly in volume during growth, the cells which surrounded the swollen part of the body were pushed aside, and commonly a circle of crushed cells surrounded

this part of the nematode (fig. 12A). This condition also occurred around egg masses (fig. 12B) and giant-cell areas (fig. 12A).

Gall formation was found to be a result of a combination of different effects. These were the increased number and volume of the affected root cells and of the maturing nematodes and their egg masses. Not a single case of necrosis was found associated with gall formation.

Root Cavities

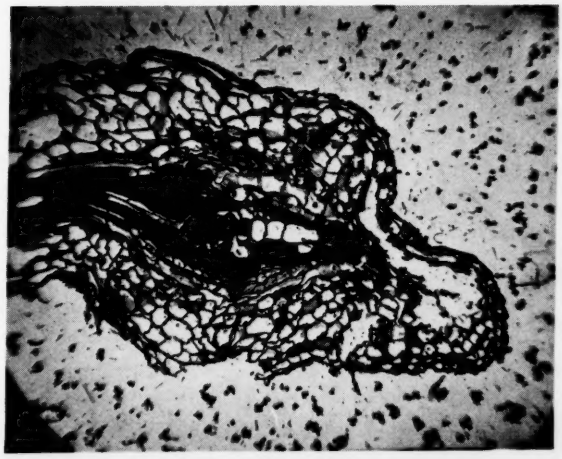
Cortical cavities or pits were found which penetrated into the vascular tissue (fig. 13). Some penetrated straight into the central core, maintaining

FIG. 10.—Knoblike structures in a giant cell.

a uniform diameter equal to that of the opening at the surface. Others expanded internally having a diameter slightly greater than that of the opening. Neither necrosis nor giant cells were seen in the surrounding tissues of these cavities.

SUITABILITY OF P.R. 980 SUGARCANE AS A HOST

The percentage of mature females to the total number of nematodes within the roots was used as a criterion of host suitability for reproduction of each of the five nematode isolates. The following percentages were calculated on nematode data for the entire 7-cm. root lengths studied in the primary root samples (tables 1–5), where sufficient quantities of mature females were found. Sixteen and seven-tenths percent of all the *M. arenaria* nematodes had reached maturity at the time of root harvest, which was



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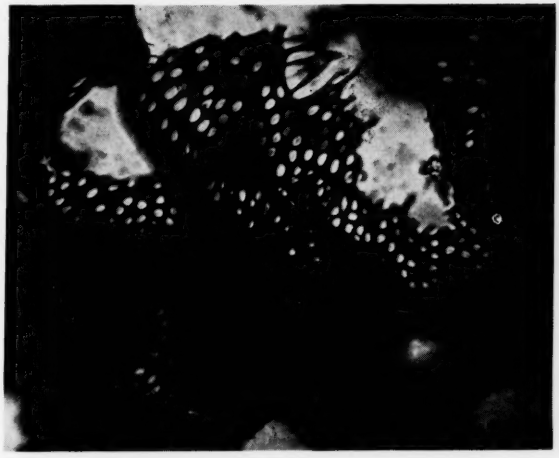

B

FIG. 11. A, Absence of apical meristem and root cap in a highly infected root tip; B, abnormality of the xylem associated with nematode injury.

5 months after inoculation. The percentage of *M. incognita incognita* that reached maturity was 6.6. Only 4.9 percent of the *M. incognita acrita* selection from pineapple reached maturity as compared to 26.3 percent of this subspecies selection from *E. foetidum* and 32.2 percent for the sub-

A

B

FIG. 12.—A, Mechanical injury to cells surrounding the posterior portion of female nematodes and the giant-cell area; B, mechanical injury to cells surrounding egg masses.

species from cucumber. Thus, there appeared to be distinct host-suitability differences for the three nematode species tested and especially for the *M. incognita acrita* selection from pineapple as compared to the other two selections of this subspecies. *M. incognita acrita* from pineapple differed

from the others also in its comparatively lower number of nematodes infecting the sugarcane (table 7). There were no previous indications of declining infectivity or viability in any of the selections during the course of culturing and at the time of inoculation of the sugarcane.

The low host-suitability rating for *M. incognita incognita* appeared to be due to a slow rate of development rather than to a low amount of infection, as the numbers of young females in the primary roots were quite high (table 2).

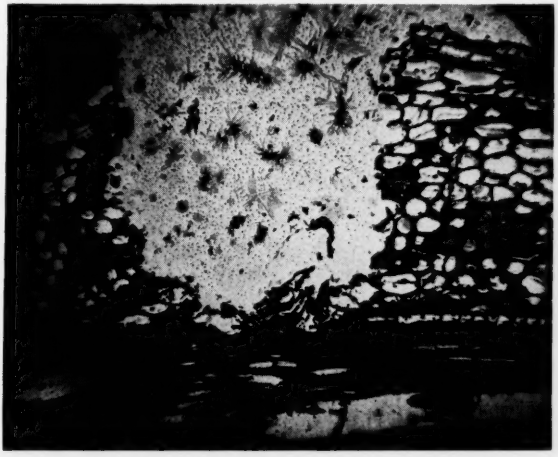

FIG. 13.—Longitudinal section of a cortical cavity.

DISCUSSION

Roots inoculated with each of the five root-knot nematode isolates developed distinct curvatures. In comparison, the noninoculated control roots were found to be straight or with a very slight wavy appearance. The internal cause of this curvature was not established. Perhaps it can serve as an additional useful field symptom for detecting the disease.

Nematodes were not solely responsible for the superficial, brownish-black lesions found on the roots, since these lesions were also found on the supernatant treated and control roots. Although the frequency of lesions was slightly higher on the roots infected with nematodes, differences in lesion appearances were not established. It is remarkable that other microorganisms were not present in these lesions, although it could be that the staining technique used would not reveal them. Since the soil was not checked for other casual agents, the presence of other soil organisms, like arthropods, was possible.

The internal lesions or cortical cavities of the roots were not solely dependent upon the presence of the nematodes. Although a definite increase in the number of cavities was found in the nematode treatments, their appearances did not differ from cavities in the control roots. It was noted that no cavities contained nematodes or the remains of their bodies, nor were giant cells formed in the immediate vicinity. This was carefully checked because Treub (*21*) had reported that cavities resulted where female root-knot nematodes had been and that giant cells could be found. Thus, these new data indicate that the cortical cavities or pits can be caused in other, as yet, unexplained ways. Loss of lateral roots was one possibility considered. However, no traces were found in the main vascular cylinder suggestive of the previous existence of lateral roots. These cavities by themselves are possibly of little harm to the roots as they do not interfere much with the vascular tissues. Perhaps, they are more significant as portals of entry for various organisms.

The observation that large numbers of nematodes in all stages of development were present in the first centimeter of the roots can be explained as the result of retardation of root growth in length, since infection did not occur in portions of the roots where differentiation was already completed. The internal study of roots having galls at the apex showed that the apical meristem and the root cap were either very much reduced in size or completely absent. Such roots would be expected to grow slowly or not at all. This effect may also be caused by the penetration of large numbers of larvae through the apical meristem, and, even though a gall is not found present at the tip, root growth is expected to be retarded or stopped.

Retardation or stoppage of root growth could be an important factor to the plant since short roots furnish less support for the plant and less surface area for contact with the mineral elements and moisture in the soil. In addition to this, a gall in the root may represent a partial or complete disruption of the vascular tissue. These effects could account for symptoms such as chlorosis, dwarfing, wilting, and reduction of yield.

The larger the number of nematodes and the longer they have been present in a gall, the larger the size of the gall. The spheroidal and ellipsoidal gall shapes were found to be due to the nematode distribution inside the gall. Nematodes tightly packed in a common place in the root produced a spheroidal gall. On the other hand, if these nematodes were more dispersed, but still in a small region, an ellipsoidal gall resulted. Proliferation of lateral roots in the vicinity of the galls was not a regular occurrence.

The internal study of the infected roots showed that the typical giant-cell formation occurred in sugarcane in response to all five isolates of root-knot nematodes feeding on the root tissues. In sugarcane, the giant cell is a syncytium resulting from cell enlargement, cell-wall dissolution, coales-

cence of cytoplasmic contents, and cell division. These results show partial agreement with the reports of Christie (*4*) for tomato roots, but are completely different from what Kostoff and Kendal (*11*) found for tobacco roots. The fusion of the pear-shaped nuclei of the giant cell and the presence of the knoblike structures reported by Krusberg and Nielsen (*12*) in sweetpotato roots, were also found to occur in sugarcane roots. These knoblike projections were found on the inner surfaces of giant cells in the vicinity of the vascular cylinder. The staining reaction of these projections was the same as for the walls of the giant cells, but different from the lignified xylem elements which stained red.

In this study, in which only one host was used, the giant-cell and galling responses of the host to the five root-knot nematode isolates were identical. The evidence indicated that giant-cell formation occurred in cells which had not yet become differentiated. There was no conclusive evidence that cells which have already differentiated can become transformed into giant cells. The finding of giant cells in already differentiated tissues is no proof that these cells had developed as a result of later infection. Giant-cell formation was first detectable at the beginning of the region of cell elongation. As a result of root development, this area, in which the nematodes had become sedentary and in which giant cells continued to develop, eventually became incorporated into the zone of differentiation. This explains why the nematodes may be found either completely inside or with their heads inserted into the vascular tissue. This also explains what appears to be the interruption of the normal differentiation of the vascular elements, pericycle, and endodermis by the presence of giant cells and nematodes. Thus, the position taken by the nematodes in the region of cell elongation ultimately decides which tissues are subsequently to be affected by the nematodes.

The disorderly arrangement of the xylem elements which was always found around the outside of the giant-cell area seemed also to be a response to the nematodes. It may be that these cells were partly disorganized and failed to form normal xylem tissue, although able to continue to differentiate. Cells of the root tissues were also affected mechanically by the growth of the nematode's body and by the increase in volume of the egg masses and giant cells.

The sugarcane variety P.R. 980 may be considered a host with variable suitability for the nematodes tested. This variety was unsuitable for *M. incognita acrita* from pineapple which differed markedly from the other two selections of this subspecies both in infectivity and the ability to complete maturation. This suggests the existence of strain differences in this subspecies group. The variety P.R. 980 was also of low suitability for the reproduction of *M. incognita incognita*, but this was due to a slower matur-

ation rate. These differences in host suitability may have practical value in terms of rotation practices used in growing sugarcane, but yield tests will be necessary to determine whether differences with respect to the root-knot nematode species and their possible strains will be of importance to the sugarcane industry.

SUMMARY

An experiment was conducted to determine the nature of the pathogenic effects of five isolates of root-knot nematodes on the roots of the promising commercial sugarcane variety, Puerto Rico 980, the suitability of this sugarcane as a host for the five nematodes, and the possibility of the existence of strains in the species *M. incognita acrita*.

The five root-knot nematodes, which included three species, were isolated as single egg masses from different kinds of host plants. The isolates included the species *Meloidogyne arenaria* from pepper, *M. incognita incognita* from tobacco, and three selections of *M. incognita acrita* from pineapple, *Eryngium foetidum*, and cucumber. Treatments consisted of inoculating the roots of P.R. 980, grown in steam-sterilized soil, with suspensions of 5,000 root-knot nematode larvae and, as a separate treatment, with the supernatant water from about the nematodes. Control plants were not inoculated. After a period of 5 months the roots were harvested and fixed in formalin-aceto-alcohol. The roots were studied to determine the gross effects of the nematodes on the roots, the number of nematodes present and their life cycle stages within the roots, and their pathogenic effects.

Larvae penetrated mostly through the apical meristem of the root tip and migrated to the central part of the root, leaving galleries or burrows composed of broken and separated cells.

The internal response of the root tissues to the five nematodes was mainly the formation of giant cells. No necrosis was observed. The first indication of the formation of giant cells was a slight increase in size of the cells and of their nuclei. This apparently was followed by cell-wall dissolution and coalescence of cytoplasmic contents resulting in a multinucleate syncytium known as a giant cell. Groups of small numbers of nuclei within the giant cell fused together. Cell division occurred in the cells adjacent to the giant cells but the walls of these cells also became dissolved and their contents were gradually incorporated by the giant cells. Groups and individual nuclei then became flask-shaped and aggregated with their narrow ends oriented toward a central point. Finally, their membranes dissolved and their contents coalesced.

Giant cells were first detected at the beginning of the region of cell elongation and apparently were formed only from cells which had not differentiated. However, as a result of root development, giant cells were

also found in and around the vascular cylinder and gave the impression that the nematodes had modified already differentiated tissues.

Xylem elements, which probably had already begun differentiation at the time of infection, completed development but failed to form a normal tissue in proximity to the developing nematodes.

Mechanical damage also occurred in the root cells as a result of pressure from expansion in volume on the giant cells, maturing nematodes, and egg masses. The galled areas of the roots also resulted from these expansions. Spheroidal galls resulted when the nematodes within them were close together, while ellipsoidal galls formed when the nematodes were more widely dispersed.

Meristems and root caps of highly infected roots were much reduced or absent, resulting in a high percentage of galls and nematodes at or close to the apexes. In general, the highest number of nematodes per centimeter of root occurred in the first centimeter from the root apex.

Lateral root proliferation was not found to be consistently associated with gall formation. Pronounced root curvature was associated with nematode infection. Superficial lesions on the roots and cortical cavities were not produced solely by the nematodes, and although their frequency was higher on nematode-infected roots, differences in appearance to those on the control were not found. No cavities contained nematodes, remains of their bodies, nor were giant cells formed in the immediate vicinity.

This sugarcane variety, P.R. 980, was an unsuitable host for one *M. incognita acrita* selection, which differed enough in its infectivity and maturation as to suggest a strain difference. Reproduction of *M. incognita incognita* was also low, but evidently due to a slow maturation rate as its infection rate was high. This nematode would probably increase in population during the long growing period of sugarcane in the field. P.R. 980 was a suitable host permitting large population increases for *M. arenaria* and for two of the *M. incognita acrita* selections. Pathogenic relationships of the five nematode isolates were similar in other aspects in this host.

RESUMEN

Se llevó a cabo un experimento con el fin de determinar la naturaleza de los efectos patogénicos de cinco tipos diferentes de nematodos de agalla a las raíces de la prometedora variedad comercial de caña Puerto Rico 980; para estudiar la adaptabilidad de la mencionada variedad de caña como planta hospedadora de estos tipos de nematodos; y para conseguir evidencia de que hubieran distintas razas de la especie *M. incognita acrita*.

A los cinco tipos de nematodos, de tres especies, procedentes de diferentes plantas hospedadoras, se les separaron sus sacos de huevos. Estos tipos incluyeron las siguientes especies: *Meloidogyne arenaria* del pimiento, *M.*

incognita incognita del tabaco y tres selecciones de *M. incognita acrita* de piña, de culantro del monte (*Eryngium foetidum*) y de pepinillo.

Los tratamientos consistieron en inocular las raíces de la caña P.R. 980, desarrolladas en suelo esterilizado a vapor, con suspensiones de 5,000 larvas de nematodos de agalla y también como un tratamiento aparte, aplicándoles el agua que flotaba alrededor de los nematodos. El tercer tratamiento era el testigo. Después de cinco meses, las raíces se extrajeron del suelo y se fijaron en una solución de formalina, ácido acético y alcohol. Luego se estudiaron para determinar cómo las afectaban los nematodos, cuántos de éstos había presente y en qué etapa en su ciclo de vida se hallaban.

Se observó que las larvas de los nematodos penetraron principalmente a través del meristemo apical de la raíz y migraron a la parte central de ésta, dejando galerías compuestas de células rotas y separadas.

Se descubrieron células gigantes donde empezaba la región de alargamiento celular y, aparentemente, éstas se formaron de células que no se habían diferenciado. Sin embargo, como resultado del crecimiento radical, también se encontraron células gigantes dentro y alrededor del cilindro vascular, lo cual daba la impresión de que los nematodos habían modificado el tejido ya diferenciado.

La reacción de los tejidos internos radicales a la presencia de los cinco tipos de nematodos fue principalmente la formación de células gigantes y no se observó necrosis alguna. La primera indicación de la formación de células gigantes fue que hubo un pequeño aumento en el tamaño de las células y sus núcleos. Esto aparentemente fue seguido por la disolución de las paredes celulares y la coalescencia de los contenidos citoplásmicos, de lo cual resultó un sincitio multinucleado conocido como célula gigante. Algunos grupos pequeños de núcleos, dentro de las células gigantes, se unieron. Ocurrió división celular en las células adyacentes a las células gigantes, pero las paredes de éstas también fueron disueltas y sus contenidos fueron gradualmente incorporados por las células gigantes. Otros grupos de núcleos y núcleos individuales luego se tornaron piriformes y se agruparon poniendo sus extremos más angostos orientados hacia un punto central. Finalmente, sus membranas se disolvieron y sus contenidos se fundieron.

Los componentes del xilema, que probablemente habían ya empezado a diferenciarse al tiempo de la infección, completaron su desarrollo; pero no formaron un tejido normal en las inmediaciones de los nematodos según se desarrollaban.

Ocurrió un daño mecánico a las células radicales como resultado de la presión ejercida por la expansión en volumen de las células gigantes, de los nematodos que estaban desarrollándose y de la de los sacos de huevos. También se formaron áreas hipertrofiadas en las raíces como resultado de

estas expansiones. Además, se formaron agallas esferoidales cuando los nematodos dentro de éstas se encontraban juntos, mientras que por otra parte, se desarrollaron agallas elipsoidales cuando los nematodos se encontraban dispersos.

Los meristemos y las pilorrizas de raíces altamente infectadas se redujeron considerablemente o desaparecieron, lo que resultó en una alta acumulación de agallas y nematodos en, o cerca de los ápices. En general, se observó que el número más alto de nematodos por centímetro de raíz se concentró en el primer centímetro apical.

La proliferación de raíces laterales no estaba asociada consistentemente a la formación de agallas. La distorción de las raíces se encontró asociada a la infección de los nematodos. Las lesiones superficiales en las raíces y cavidades corticales no las produjeron únicamente los nematodos y aunque esta condición abundaba más en las raíces infectadas, no surgieron diferencias al compararse con las que se observaron en los testigos. Tampoco se observaron nematodos, ni residuos de sus cuerpos en las cavidades, ni tampoco se formaron células gigantes en sus alrededores.

La variedad de caña de azúcar P.R. 980, no es una planta hospedadora propicia para una de las selecciones de *M. incognita acrita*, porque difería lo suficiente en su infectividad y desarrollo como para sugerir una raza diferente. La reproducción de *M. incognita incognita* fue también poca, evidentemente debido a un lento proceso de madurez, toda vez que su grado de infeción era considerable. Este nematodo probablemente pudo aumentar en número durante el largo período de crecimiento de la caña de azúcar en el campo. La variedad P.R. 980 fue una planta hospedadora propicia, permitiendo grandes aumentos en número de *M. arenaria* y dos selecciones de *M. incognita acrita*. Las relaciones patógenicas de los cinco tipos de nematodos fueron similares en otros aspectos en cuanto a esta planta hospedadora.

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Effects of Irrigation at Different Growth Stages, and of Nitrogen Levels on Corn Yields in Lajas Valley, P.R.[1]

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INTRODUCTION

Some years ago concern was expressed over the rate at which new lands were being developed for agriculture. Recently there has been a change in thinking and the new limit to expansion is not land, but water. Agricultural production can be increased to a maximum on lands already developed by a proper combination of irrigation with other improved cultural practices.

Competition for available water by industries, cities, and farms, and the relatively large quantity of water needed for agriculture justify studies to improve the efficiency of water use in agriculture. The limit of water-application efficiency can be reached only when a proper evaluation of plant needs is available. Inadequate soil moisture at critical stages of crop growth often limits crop production. Some studies have been made in the United States by Robins and Domingo (*4*)[3] wherein they found that moisture depletion to near the wilting percentage by field corn at certain physiologic growth stages markedly reduced grain yields. In Mexico, Fernández and Laird (*2*) concluded on the basis of their data that moisture deficits during the tasseling stage reduced corn yields significantly. Since climatic and soil conditions may modify the results obtained under different conditions, two experiments were conducted at the Lajas Substation to evaluate the effect of irrigation at different growth stages on the production of field corn in the Lajas Valley.

EXPERIMENTAL PROCEDURE

The first experiment was planted March 16, 1957 and harvested July 22, 1957; the second was planted April 15, 1958 and harvested August 19, 1958. A cubic lattice design was used in both experiments. The treatments included a combination of 9 irrigation levels and 3 nitrogen levels, with 4 replications, on a total of 108 plots. A uniform population of 19,400 plants

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[3] Italic numbers in parentheses refer to Literature Cited, p. 105.

per acre was used. A field-corn variety, Mayorbela, was planted with 3 feet between rows and 9 inches between plants. Two seed were placed in each hole, thinning to one plant per hole after the plants emerged. The plots were 6 rows (18 feet) wide and 18 feet long. The entire experiment covered an area of about 240 feet wide by 180 feet long.

The soil used was classified as Santa Isabel clay. This is one of the predominant soil types in Lajas Valley. No crops had been planted on this site during the last few years.

The soil moisture retained at different tensions and other physical properties were determined (table 1).

TABLE 1.—*Moisture-release data (percentage by weight) for Santa Isabel clay soil, Lajas, P.R.*

Moisture tension (atmospheres)	Soil moisture at—	
	Soil depth of 3 to 6 inches	Soil depth of 18 to 21 inches
0.1	41.3	43.7
.3	38.3	39.7
.6	37.2	37.3
1.0	35.8	35.2
2.0	34.3	34.0
15.0	21.4	22.7
Bulk density[1]	1.03	1.24

[1] Grams per cubic centimeter.

IRRIGATION

The following irrigation treatments were tested:

No. 1. Irrigated when 20 percent of the available moisture had been depleted from the active root zone.

No. 2. Irrigated when 60 percent of the available moisture had been depleted from the active root zone.

No. 3. Not irrigated after the crop was established.

No. 4. Irrigated as in treatment No. 1 until hard-dough stage; no irrigation thereafter.

No. 5. Irrigated as in treatment No. 1 until silking stage; no irrigation thereafter.

No. 6. Irrigated as in treatment No. 1 until tasseling stage; no irrigation thereafter.

No. 7. Not irrigated after the crop was established until hard-dough stage and as in treatment No. 1 thereafter.

No. 8. Not irrigated after the crop was established until silking stage and as in treatment No. 1 thereafter.

No. 9. Not irrigated after the crop was established until tasseling stage and as in treatment No. 1 thereafter.

The soil was plowed and harrowed several times until a good seedbed was obtained. The whole area was divided into 108 plots 20 feet wide by 20 feet long by means of ditches made around each plot. A basin was made of each plot by means of border ridges about 8 inches high. The plot (basin) was filled with water at each irrigation by means of fire hoses that were connected to the irrigation pipelines. A heavy irrigation was applied to the whole area after each experiment was planted. Subsequent irrigations were made according to the treatments involved.

FERTILIZER

The nitrogen-fertilizer treatments used were 0, 60, and 120 pounds of nitrogen per acre as ammonium sulfate (20.5-percent N) and were applied when the corn plants were about 8 inches high. Phosphorus and potassium in the form of superphosphate (20.5-percent P_2O_5), and muriate of potash (61-percent K_2O), respectively, were applied to the whole experiment at the rate of 200 pounds each of P_2O_5 and K_2O per acre. All the fertilizer was placed in a small furrow opened about 3 inches on both sides of the corn plant.

SOIL-MOISTURE CONTROL

Disturbed and undisturbed soil samples were taken from 3 to 6 and from 18 to 21 inches deep at the site where the experiments were conducted. The soil moisture retained by the undisturbed soil samples at different tensions in the low-tension range was determined in the pressure plate. The moisture retained by the disturbed soil samples at higher tensions was determined in the pressure membrane apparatus (*5*). Bulk-density determinations were made at each depth.

Tensiometers (*3*) were installed close to the corn plants in the highest fertility plots with irrigation treatment No. 1 at 6-, 12-, 18-, and 24-inch depths. In the plots with irrigation treatments Nos. 7, 8, 9 a tensiometer was installed at a depth of 12 inches in a high-fertility plot. Readings of the instruments were taken every day.

Irrigation of the plots under irrigation treatment No. 1 were made when the average soil-moisture tensions in the active root zone became 750 cm. of water. Gypsum resistance-blocks of the Bouyoucos type (*1*) and custom-made models were installed in the second experiment at 6-, 12-, 18-, 24-, 30-, and 36-inch depths in the plots under irrigation treatments Nos. 1, 2, and 3. The irrigations of the plots under irrigation treatment No. 2 were

made when the average available soil moisture in the active root zone dropped to 28.8 percent. About 2 inches of water were applied whenever

TABLE 2.—*Irrigation frequencies used in the corn experiments at Lajas Substation*

Dates of irrigation for—	Moisture treatment No.—[1]								
	1	2	3	4	5	6	7	8	9
Experiment No. 1, 1957:									
Mar. 18–19	×	×	×	×	×	×	×	×	×
Apr. 3–4	×	×	—	×	×	×	—	—	—
Apr. 16–17	×	×	—	×	×	×	—	—	—
May 1	×	—	—	×	×	×	—	—	—
May 7–8	×	×	—	×	×	×	—	—	—
May 15–16	×	—	—	×	×	—	—	—	—
May 24–26	×	×	—	×	—	—	—	×	×
June 7–8	×	—	—	—	—	—	×	×	×
June 14–15	×	×	—	—	—	—	×	×	×
June 25–26	×	—	—	—	—	—	×	×	×
Total	10	6	1	7	6	5	4	5	5
Experiment No. 2, 1958:									
Apr. 16–17	×	×	×	×	×	×	×	×	×
May 20–21	×	×	—	×	×	×	—	—	—
May 27–28	×	—	—	×	×	×	—	—	—
June 18–19	×	—	—	×	—	—	—	×	×
July 16–17	×	—	—	—	—	—	×	×	×
Total	5	2	1	4	3	3	2	3	3

[1] Irrigation treatments were as follows: 1, Irrigated when 20 percent of the available moisture had been depleted from the active root zone. 2, Irrigated when 60 percent of the available moisture had been depleted from the active root zone. 3, Not irrigated after the crop was established. 4, Irrigated as was treatment No. 1 until hard-dough stage; no irrigation thereafter. 5, Irrigated as was treatment No. 1 until silking stage; no irrigation thereafter. 6, Irrigated as was treatment No. 1 until tasseling stage; no irrigation thereafter. 7, Not irrigated after the crop was established until hard-dough stage; like treatment No. 1 thereafter. 8, Not irrigated after the crop was established until silking stage; like treatment No. 1 thereafter. 9, Not irrigated after the crop was established until tasseling stage; like treatment No. 1 thereafter. × means one irrigation.

an irrigation was performed (table 2). Soil samples for moisture determination were taken during the whole growing season to indicate the irrigation of the plots under treatment No. 2 and to calculate the moisture extracted from the root zone in each moisture treatment.

In experiment No. 1 the soil samples were taken with a soil auger at the following depths: 0 to 9, 9 to 15, 15 to 21, 21 to 27, 27 to 33, and 33 to 39 in ches. In experiment No. 2 duplicate soil samples were taken at three depths: 3 to 9, 15 to 21, and 27 to 33 inches. The soil samples were taken twice a week in the plots under a fertilizer application of 0, 60, and 120 pounds of nitrogen per acre with irrigation treatments Nos. 1, 2, and 3, respectively. Soil samples were taken in the plots under treatments Nos. 4, 5, 6, 7, 8, and 9 when their initial irrigation treatments were changed.

All samples were weighed and placed in an oven for 24 hours at 105°C., and the percentage of moisture was determined on an oven-dry-weight basis. The water extracted from the top 3 feet of soil in the plots with each irrigation treatment was calculated for short periods throughout the whole growing season. A total of six samples under each moisture treatment was used to calculate the water extracted during those short periods and throughout the whole growing season. The consumptive use of water was calculated by adding the rainfall to the water extracted during those periods.

CULTURAL PRACTICES

Weedings were made as necessary to maintain the plots free of weeds. Every 10 days the whole experiment was sprayed with DDT (50-percent wettable) at the rate of 4 pounds per 100 gallons of water to control worm attack. When the kernels were dry, the central 12-foot-long sections of the central four rows of each plot were harvested. Percentage moisture of the kernel was determined.

EXPERIMENTAL RESULTS

An evaluation was made of the soil-moisture data and the effects of irrigation at different growth stages on corn yields.

SOIL MOISTURE

The rainfall distribution during the growth period of each experiment is shown in table 3. As can be calculated from this table, the rainfall for the March–July period in 1957 totaled 4.54 inches, which was 10.73 inches below the 10-year average for Lajas. However, in 1958, the April–August period totaled 27.06 inches which was 9.10 inches above the average. In that period only the rainfall in May was below the average.

The consumptive use of water during short periods of time and throughout the whole growing season of both experiments is presented in tables 4 to 14.

As shown in tables 4, 5, and 6, the total consumptive use in 125 days in the first experiment varied from 26.07 inches in the frequently irrigated plots (treatment No. 1) to 21.64 and 7.59 in the intermediately irrigated

TABLE 3.—*Inches of daily rainfall during the growth period of both experiments at Lajas, P.R., 1957–58*[1]

Date	1957					1958				
	March	April	May	June	July	April	May	June	July	August
1	0.02							0.49	0.79	
2	.14	0.12					0.13	.12	1.07	1.15
3				0.01			.28	.47		.28
4				.03	0.04		.46			
5							.10	.05	.39	2.20
6					.02	0.17	.30	.25	.40	
7							.02	1.53	1.08	
8		.02								.05
9							.23	.06	.49	.10
10						.11		.02		.22
11						.07				.10
12			0.29	.28				.75		.07
13	.05		.04			.07	.06			.10
14		.10				.03				
15						.16		.12		
16		.02	.22	.05					.20	.07
17	.09					.03				.05
18				.16		.73	.01			
19		.13		.13		1.10	.10			
20		.63				1.02		.76	.20	
21			.08						1.54	
22			.21			.23		0.7	.03	.21
23						.25				
24		.06	.04	.21	.08	.02		.05	.70	
25		.13	.48	.06		.53		.01		
26		.03								
27									.10	
28			.28				.44		.10	.60
29			.01						.02	
30			.01	.07	.20	.22	.46	.07		
31										2.60
Total	0.30	1.24	1.66	1.00	0.34	4.74	2.59	4.82	7.11	7.80
10-year (average)	2.91	2.19	2.81	2.43	4.93	2.19	2.81	2.43	4.93	5.60

[1] Data from raingage located in the northeast section of the Lajas Substation farm.

(treatment No. 2) and nonirrigated plots (treatment No. 3), respectively. Tables 7, 8, and 9 show the consumptive use of water in 125 days in the plots that were frequently irrigated from planting to the hard-dough (treatment No. 4), to the silking (treatment No. 5), and to the tasseling

stages (treatment No. 6), respectively. It varied from 23.29 inches in the plots irrigated until the hard-dough stage, to 21.79 and to 19.25 inches in the plots irrigated until the silking and tasseling stage, respectively. Tables 10 and 11 show the consumptive use of water in the plots that were frequently irrigated from the hard-dough, (treatment No. 7), the silking

TABLE 4.—*Consumptive use of water by field corn in the frequently irrigated plots throughout the whole growing season (treatment No. 1) of the first experiment, by periods, Mar. 19 to July 21, 1957*

Period	Consumptive-use interval	Total consumptive use	Average daily consumptive use
	Days	*Inches*	*Inches*
Mar. 19 to Mar. 20[1]	2	0.186	0.093
Mar. 21 to Apr. 1	12	1.113	.093
Apr. 2 to Apr. 4[1]	3	.510	.170
Apr. 5 to Apr. 11	7	1.545	.221
Apr. 12 to Apr. 22[1]	11	2.607	.237
Apr. 23 to Apr. 29	7	1.764	.252
Apr. 30 to May 2[1]	3	1.050	.350
May 3 to May 6	4	1.696	.424
May 7 to May 9[1]	3	.951	.317
May 10 to May 13	4	.709	.177
May 14 to May 16[1]	3	.825	.275
May 17 to May 23	7	2.672	.382
May 24 to May 27[1]	4	1.200	.300
May 28 to June 6	10	1.561	.156
June 7 to June 10[1]	4	1.008	.252
June 11 to June 13	3	.894	.298
June 14 to June 17[1]	4	.948	.237
June 18 to June 24	7	.948	.135
June 25 to June 27[1]	3	.411	.137
June 28 to July 18	21	3.021	.144
July 19 to July 21[1]	3	.450	.150
Total	125	26.069	

[1] Consumptive-use data calculated by inter- and extrapolation.

(treatment No. 8), and tasseling stages (treatment No. 9) to harvesttime. As can be observed the total consumptive use in 125 days was of 13.25 inches in the plots frequently irrigated after the hard-dough stage, and 13.62 in those plots frequently irrigated after the silking and tasseling stages. The consumptive use of water in 125 days in the second experiment is presented in tables 12, 13, and 14. It was 30.20 inches in the frequently irrigated plots throughout the whole growing season (treatment No. 1) and in the frequently irrigated plots until the hard-dough stage (treatment No. 4); 23.50 in the intermediately irrigated (treatment No. 2), the

frequently irrigated until the silking (treatment No. 5), and the tasseling stages (treatment No. 6); and 21.47 in the nonirrigated plots (treatment No. 3), the frequently irrigated plots after the tasseling (treatment No. 9), silking (treatment No. 8), and hard-dough stages (treatment No. 7).

In the intermediately irrigated plots (treatment No. 2) of the first experiment conducted, the average consumptive use of water during the first

TABLE 5.—*Consumptive use of water by field corn in the intermediately irrigated plots throughout the whole growing season (treatment No. 2) of the first experiment, by periods, Mar. 19 to July 21, 1957*[1]

Period	Consumptive-use interval	Total consumptive use	Average daily consumptive use
	Days	*Inches*	*Inches*
Mar. 19 to Mar. 20[2]	2	0.186	0.093
Mar. 21 to Apr. 1	12	1.113	.093
Apr. 2 to Apr. 4[2]	3	.510	.170
Apr. 5 to Apr. 11	7	1.545	.221
Apr. 12 to Apr. 22[2]	11	2.607	.237
Apr. 23 to Apr. 29	7	1.764	.252
Apr. 30 to May 6	7	1.286	.184
May 7 to May 9[2]	3	.669	.223
May 10 to May 13	4	.990	.248
May 14 to May 23	10	1.859	.186
May 24 to May 27[2]	4	1.028	.257
May 28 to June 6	10	3.211	.321
June 7 to June 13	7	1.259	.179
June 14 to June 17[2]	4	.572	.143
June 18 to June 27	10	.978	.098
June 28 to July 18	21	1.833	.087
July 19 to July 21[2]	3	.228	.076
Total	125	21.638	

[1] Consumptive-use data from Mar. 19 to Apr. 29 are the same as for the frequently irrigated, plots. All soil-moisture samples taken under each moisture treatment were combined to calculate the moisture extracted during that period.

[2] Consumptive-use data calculated by inter- and extrapolation.

52 days after planting was 0.186 inches per day, increasing to an average of 0.253 inches per day during the period of 53 to 80 days after planting, and decreasing to an average of 0.108 inch daily from 81 to 125 days after planting (derived from data in table 5). In the second experiment, as derived from data in table 14, the average water use in the nonirrigated plots (treatment No. 3) during the first 58 days after planting was 0.165 inches per day, increasing to 0.292 inch per day from 59 to 77 days after planting, and decreasing to 0.132 inch daily from 78 to 125 days after planting.

TABLE 6.—*Consumptive use of water by field corn in the nonirrigated plots throughout the whole growing season (treatment No. 3) of the first experiment, by periods, Mar. 19 to July 21, 1957*[1]

Period	Consumptive-use interval	Total consumptive use	Average daily consumptive use
	Days	*Inches*	*Inches*
Mar. 19 to Mar. 20[2]	2	0.186	0.093
Mar. 21 to Apr. 1	12	1.113	.093
Apr. 2 to Apr. 11	10	.757	.076
Apr. 12 to Apr. 29	18	1.351	.075
Apr. 30 to May 6	7	.604	.086
May 7 to May 13	7	.387	.055
May 14 to June 6	24	1.259	.052
June 7 to June 20	14	.442	.032
June 21 to July 18	28	1.325	.047
July 19 to July 21[2]	3	.165	.055
Total	125	7.589	

[1] Consumptive-use data from Mar. 19 to Apr. 1 are the same as for the frequently and intermediately irrigated plots. All soil-moisture samples taken under each moisture treatment were combined to calculate the moisture extracted during that period.

[2] Consumptive-use data calculated by extrapolation.

TABLE 7.—*Consumptive use of water by field corn in the frequently irrigated plots until the hard-dough stage with no irrigation thereafter (treatment No. 4) for the first experiment, by periods, Mar. 19 to July 21, 1957*[1]

Period	Consumptive-use interval	Total consumptive use	Average daily consumptive use
	Days	*Inches*	*Inches*
Mar. 19 to Mar. 20[2]	2	0.186	0.093
Mar. 21 to Apr. 1	12	1.113	.093
Apr. 2 to Apr. 4[2]	3	.510	.170
Apr. 5 to Apr. 11	7	1.545	.221
Apr. 12 to Apr. 22[2]	11	2.607	.237
Apr. 23 to Apr. 29	7	1.764	.252
Apr. 30 to May 2[2]	3	1.050	.350
May 3 to May 6	4	1.696	.424
May 7 to May 9[2]	3	.951	.317
May 10 to May 13	4	.709	.177
May 14 to May 16[2]	3	.825	.275
May 17 to May 23	7	2.672	.382
May 24 to May 27[2]	4	1.268	.317
May 28 to June 10	14	3.048	.218
June 11 to July 18	38	3.104	.082
July 19 to July 21[2]	3	.246	.082
Total	125	23.294	

[1] Consumptive-use data from Mar. 19 to May 23 are the same as for the frequently irrigated plots (treatment No. 1).

[2] Consumptive-use data calculated by inter- and extrapolation.

TABLE 8.—*Consumptive use of water by field corn in the frequently irrigated plots until the silking stage with no irrigation thereafter (treatment No. 5) for the first experiment, by periods, Mar. 19 to July 21, 1957*[1]

Period	Consumptive-use interval	Total consumptive use	Average daily consumptive use
	Days	*Inches*	*Inches*
Mar. 19 to Mar. 20[2]	2	0.186	0.093
Mar. 21 to Apr. 1	12	1.113	.093
Apr. 2 to Apr. 4[2]	3	.510	.170
Apr. 5 to Apr. 11	7	1.545	.221
Apr. 12 to Apr. 22[2]	11	2.607	.237
Apr. 23 to Apr. 29	7	1.764	.252
Apr. 30 to May 2[2]	3	1.050	.350
May 3 to May 6	4	1.696	.424
May 7 to May 9[2]	3	.951	.317
May 10 to May 13	4	.709	.177
May 14 to May 16[2]	3	.825	.275
May 17 to May 23	7	2.672	.382
May 24 to June 10	18	3.486	.194
June 11 to July 18	38	2.477	.065
July 19 to July 21[2]	3	.195	.065
Total	125	21.786	

[1] Consumptive-use data from Mar. 19 to May 23 are the same as for the frequently irrigated plots (treatment No. 1).

[2] Consumptive-use data calculated by inter- and extrapolation.

TABLE 9.—*Consumptive use of water by field corn in the frequently irrigated plots until the tasseling stage with no irrigation thereafter (treatment No. 6) for the first experiment, by periods, Mar. 19 to July 21, 1957*[1]

Period	Consumptive-use interval	Total consumptive use	Average daily consumptive use
	Days	*Inches*	*Inches*
Mar. 19 to Mar. 20[2]	2	0.186	0.093
Mar. 21 to Apr. 1	12	1.113	.093
Apr. 2 to Apr. 4[2]	3	.510	.170
Apr. 5 to Apr. 11	7	1.545	.221
Apr. 12 to Apr. 22[2]	11	2.607	.237
Apr. 23 to Apr. 29	7	1.764	.252
Apr. 30 to May 2[2]	3	1.050	.350
May 3 to May 6	4	1.696	.424
May 7 to May 9[2]	3	.951	.317
May 10 to May 13	4	.709	.177
May 14 to June 10	28	4.444	.159
June 11 to July 18	38	2.477	.065
July 19 to July 21[2]	3	.195	.065
Total	125	19.247	

[1] Consumptive-use data from Mar. 19 to May 13 are the same as for the frequently irrigated plots (treatment No. 1).

[2] Consumptive-use data calculated by inter- and extrapolation.

TABLE 10.—*Consumptive use of water by field corn in the nonirrigated plots until the hard-dough stage with frequent irrigation thereafter (treatment No. 7) for the first experiment, by periods, Mar. 19 to July 21, 1957*[1]

Period	Consumptive-use interval	Total consumptive use	Average daily consumptive use
	Days	*Inches*	*Inches*
Mar. 19 to Mar. 20[2]	2	0.186	0.093
Mar. 21 to Apr. 1	12	1.113	.093
Apr. 2 to Apr. 11	10	.757	.076
Apr. 12 to Apr. 29	18	1.351	.075
Apr. 30 to May 6	7	.604	.086
May 7 to May 13	7	.387	.055
May 14 to June 6	24	1.259	.052
June 7 to June 10[2]	4	.464	.116
June 11 to June 13	3	.404	.135
June 14 to June 17[2]	4	.608	.152
June 18 to June 24	7	1.262	.180
June 25 to June 27[2]	3	.540	.180
June 28 to July 18	21	3.778	.180
July 19 to July 21[2]	3	.540	.180
Total	125	13.253	

[1] Consumptive-use data from Mar. 19 to June 6 are the same as for the nonirrigated plots (treatment No. 3).

[2] Consumptive-use data calculated by inter- and extrapolation.

TABLE 11.—*Consumptive use of water by field corn in the nonirrigated plots until the silking stage with frequent irrigation thereafter (treatment No. 8) for the first experiment, by periods, Mar. 19 to July 21, 1957*[1]

Period	Consumptive-use interval	Total consumptive use	Average daily consumptive use
	Days	*Inches*	*Inches*
Mar. 19 to Mar. 20[2]	2	0.186	0.093
Mar. 21 to Apr. 1	12	1.113	.093
Apr. 2 to Apr. 11	10	.757	.076
Apr. 12 to Apr. 29	18	1.351	.075
Apr. 30 to May 6	7	.604	.086
May 7 to May 13	7	.387	.055
May 14 to May 27[2]	14	1.050	.075
May 28 to June 13	17	1.776	.104
June 14 to June 17[2]	4	.728	.182
June 18 to June 24	7	1.580	.226
June 25 to June 27[2]	3	.606	.202
June 28 to July 18	21	3.046	.145
July 19 to July 21[2]	3	.435	.145
Total	125	13.619	

[1] Consumptive-use data from Mar. 19 to May 13 are the same as for the nonirrigated plots (treatment No. 3). The consumptive-use data throughout the whole growing season are the same as for the plots not irrigated until the tasseling stage with frequent irrigation thereafter (treatment No. 9).

[2] Consumptive-use data calculated by inter- and extrapolation.

Figures 1 and 2 show the irrigations, rainfall, and the available soil moisture in the active root zone of the plots under the different irrigation treatments during the growing season of the first experiment conducted. As shown in figure 1, there was available moisture at each horizon throughout the whole growing season in the frequently irrigated plots (treatment

TABLE 12.—*Consumptive use of water by field corn in the frequently irrigated plots throughout the whole growing season (treatment No. 1) for the second experiment, by periods, Apr. 16 to Aug. 18, 1958*[1]

Period	Consumptive-use interval	Total consumptive use	Average daily consumptive use
	Days	*Inches*	*Inches*
Apr. 16 to Apr. 21[2]	6	1.062	0.177
Apr. 22 to May 1	10	2.254	.225
May 2 to May 13	12	1.865	.155
May 14 to May 18	5	.732	.146
May 19 to May 22[2]	4	.788	.197
May 23 to May 25	3	.714	.238
May 26 to May 28[2]	3	.696	.232
May 29 to June 5	8	1.784	.223
June 6 to June 12	7	2.893	.413
June 13 to June 16	4	1.404	.351
June 17 to June 22[2]	6	2.286	.381
June 23 to July 1	9	3.801	.422
July 2 to July 14	13	2.405	.185
July 15 to July 17[2]	3	.600	.200
July 18 to July 22	5	1.030	.206
July 23 to July 28	6	1.585	.264
July 29 to Aug. 3	6	1.225	.204
Aug. 4 to Aug. 10[2]	7	1.435	.205
Aug. 11 to Aug. 17	7	1.437	.205
Aug. 18[2]	1	.205	.205
Total	125	30.201	

[1] Consumptive-use data throughout the whole growing season are the same as for the frequently irrigated plots until the hard-dough stage, with no irrigation thereafter (treatment No. 4).

[2] Consumptive-use data calculated by inter- and extrapolation.

No. 1). In the plots that were frequently irrigated until the hard-dough stage (treatment No. 4) and in which the last irrigation was applied on May 24, the available moisture decreased after May 28, reaching a low level on June 10. In the plots irrigated frequently until the silking stage (treatment No. 5), in which the last irrigation was applied on May 15, the available moisture dropped after May 17 to a critical level on June 10. In the plots irrigated frequently until the tasseling stage (treatment No. 6), to

which the last irrigation was applied on May 7, the available moisture decreased from May 10 with practically no available moisture remaining by June 6 (fig. 2). Figure 2 also shows that there was always available moisture throughout the whole growing season in the intermediately irrigated plots (treatment No. 2).

TABLE 13.—*Consumptive use of water by field corn in the intermediately irrigated plots throughout the whole growing season (treatment No. 2) for the second experiment, by periods, Apr. 16 to Aug. 18, 1958*[1]

Period	Consumptive-use data	Total consumptive use	Average daily consumptive use
	Days	*Inches*	*Inches*
Apr. 16 to Apr. 21[2]	6	1.062	0.177
Apr. 22 to May 1	10	2.254	.225
May 2 to May 13	12	1.865	.155
May 14 to May 18	5	.732	.146
May 19 to May 22[2]	4	.856	.214
May 23 to May 28	6	1.745	.291
May 29 to June 5	8	1.173	.147
June 6 to June 12	7	2.600	.371
June 13 to June 22	10	2.057	.206
June 23 to July 1	9	2.827	.314
July 2 to July 14	13	1.490	.115
July 15 to July 28	14	1.475	.105
July 29 to Aug. 10[2]	13	1.924	.148
Aug. 11 to Aug. 17	7	1.251	.179
Aug. 18	1	.193	.193
Total	125	23.504	

[1] Consumptive-use data from Apr. 16 to May 18 are the same as for the frequently irrigated plots (treatment No. 1). The consumptive-use data throughout the whole growing season are the same as for the frequently irrigated plots until the silking stage with no irrigation thereafter (treatment No. 5) and for the frequently irrigated plots until the tasseling stage with no irrigation thereafter (treatment No. 6).

[2] Consumptive-use data calculated by inter- and extrapolation.

EFFECTS OF IRRIGATION

In the second experiment there was no statistically significant difference in production due to irrigation (table 15). In the first experiment irrigation increased yields in a highly significant way (table 15). As shown in table 15, in the first experiment, the frequently irrigated plot throughout the whole growing season (treatment No. 1) outyielded in a highly significant way the nonirrigated plots (treatment No. 3), the nonirrigated plots until the hard-dough (treatment No. 7), the silking (treatment No. 8), and the tasseling stages (treatment No. 9) under the 0, 60, and 120 pounds of ni-

trogen applied per acre. However, in the plots receiving 120 pounds of nitrogen per acre, the frequently irrigated plots throughout the whole growing season (treatment No. 1) outyielded the plots frequently irrigated until the tasseling stage (treatment No. 6) at the 1-percent level of significance, and the plots frequently irrigated until the silking stage (treatment No. 5) at the 5-percent level. There was no significant difference in

TABLE 14.—*Consumptive use of water by field corn in the nonirrigated plots throughout the whole growing season (treatment No. 3) for the second experiment, by periods, Apr. 16 to Aug. 18, 1958*[1]

Period	Consumptive-use interval	Total consumptive use	Average daily consumptive use
	Days	*Inches*	*Inches*
Apr. 16 to Apr. 21[2]	6	1.062	0.177
Apr. 22 to May 1	10	2.254	.225
May 2 to May 13	12	1.865	.155
May 14 to May 18	5	.732	.146
May 19 to May 28	10	1.617	.162
May 29 to June 5	8	1.065	.133
June 6 to June 12	7	.989	.141
June 13 to June 22	10	2.892	.289
June 23 to July 1	9	2.662	.296
July 2 to July 14	13	1.026	.079
July 15 to July 28	14	2.112	.151
July 29 to Aug. 10	13	1.976	.152
Aug. 11 to Aug. 17	7	1.062	.152
Aug. 18[2]	1	.154	.154
Total	125	21.468	

[1] Consumptive-use data from Apr. 16 to May 18 are the same as for the frequently irrigated plots (treatment No. 1). The consumptive-use data throughout the whole growing season are the same as for the nonirrigated plots until the hard-dough stage (treatment No. 7), nonirrigated plots until the silking stage (treatment No. 8), and the nonirrigated plots until the tasseling stage (treatment No. 9).

[2] Consumptive-use data calculated by extrapolation.

production between the plots frequently irrigated throughout the whole growing season (treatment No. 1), the plots frequently irrigated until the hard-dough stage (treatment No. 4), and the intermediately irrigated plots (treatment No. 2).

As can be observed in table 15 and in figures 3 and 4, the difference in production between the frequently irrigated plots throughout the whole growing season (treatment No. 1) and those receiving irrigations after the corn reached the tasseling (treatment No. 9), silking (treatment No. 8), and hard-dough stages (treatment No. 7), was higher than between the

frequently irrigated plots throughout the whole growing season (treatment No. 1) and those receiving irrigation until the tasseling (treatment No. 6), silking (treatment No. 5) and hard-dough stages (treatment No. 4). As

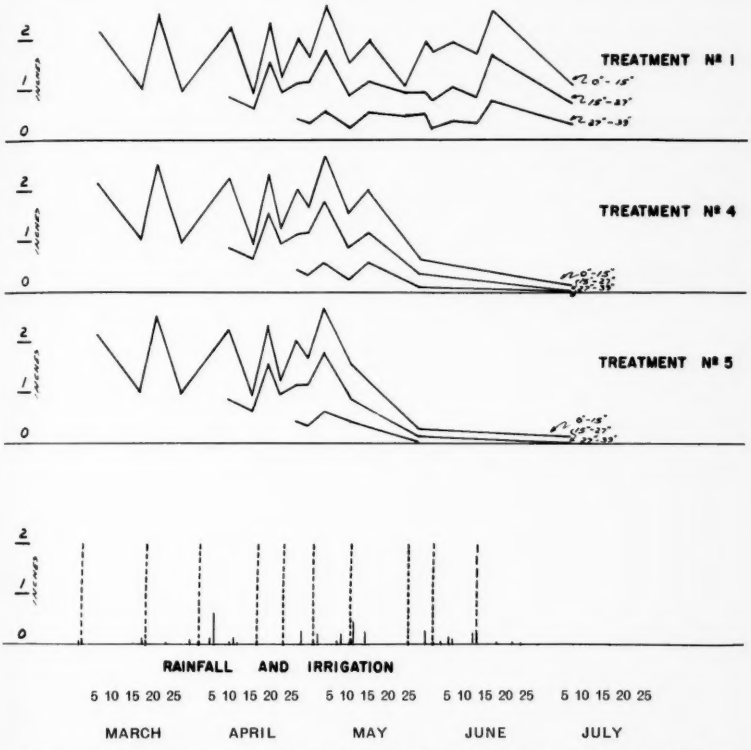
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FIG. 1.—Available water as indicated by soil sampling, in the 0 to 15-, 15 to 27-, and 27 to 39-inch horizons of the frequently irrigated plots throughout the whole growing season (top), the frequently irrigated plots until the hard-dough stage (treatment No. 4), and the frequently irrigated plots until the silking stage (treatment No. 5). The rainfall (heavy lines) and the irrigations applied (broken lines) are shown below.

derived from data of table 15, there was a reduction in yields of 12.91, 9.28, and 6.35 hundredweights per acre in the plots where the irrigation was discontinued at the tasseling (treatment No. 6), silking (treatment No. 5), and hard-dough stages (treatment No. 4), respectively.

Table 15 also shows that nitrogen increased the corn yields in the irrigated plots.

DISCUSSION

Soil-moisture deficits from the tasseling to the hard-dough stages definitely had an influence on corn-yield reductions. There was a significant reduction

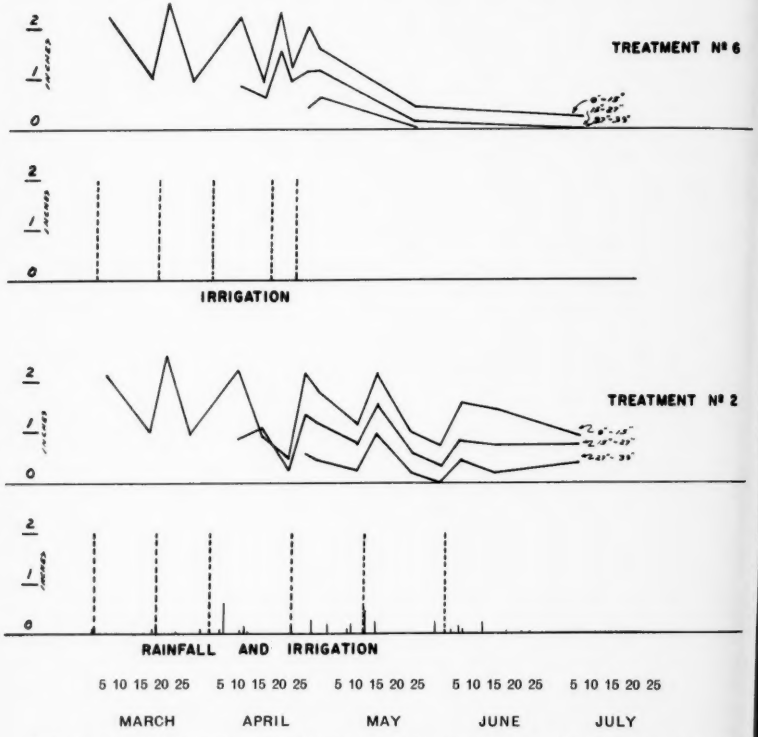
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FIG. 2.—Available water as indicated by soil sampling, in the 0 to 15-, 15 to 27-, and 27 to 39-inch horizons of the frequently irrigated plots until the tasseling stage (top) and the intermediately irrigated plots (treatment No. 2). The central chart presents the irrigations applied to the frequently irrigated plots until the tasseling stage (top figure). The bottom chart presents the rainfall (heavy lines) and the irrigations (broken lines) applied to the intermediately irrigated plots (treatment No. 2).

in yield of 22.5 and 16.2 percent in the plots where the irrigations were discontinued at the tasseling and silking stage, respectively. Robins and Domingo (*4*) found that soil-moisture deficits for periods of 1 or 2 days during the tasseling or pollination period resulted in as much as 22-percent yield reductions.

As in other corn experiments conducted during the same period (*6*), the

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Erratum An erroneous placement of the date scale on figures 1 and 2 of vol. 45, No. 2, necessitated the reprinting of these figures. This sheet should be substituted for pages 99 and 100 of the April issue.

frequently irrigated plots throughout the whole growing season (treatment No. 1) and those receiving irrigation until the tasseling (treatment No. 6), silking (treatment No. 5) and hard-dough stages (treatment No. 4). As

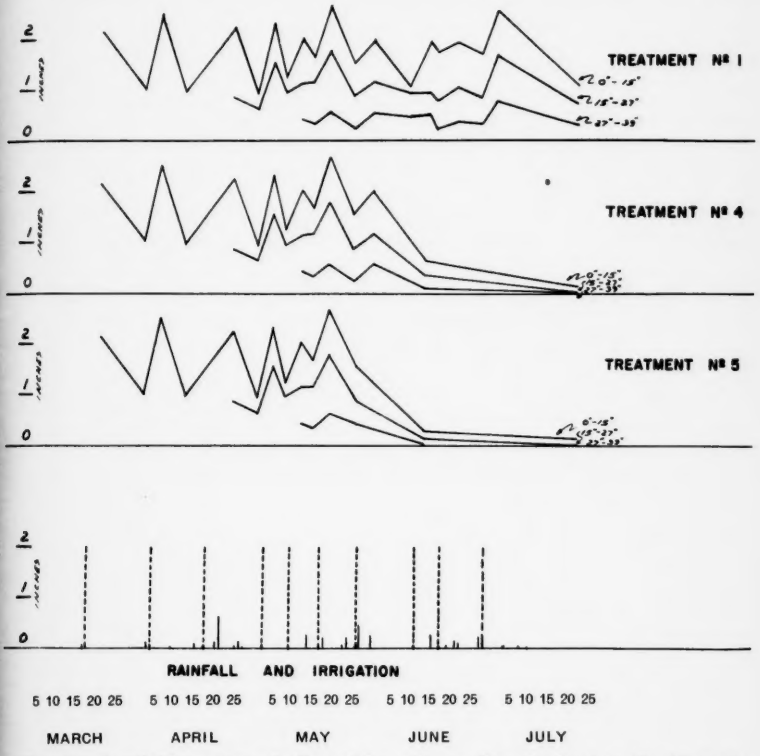
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FIG. 1.—Available water as indicated by soil sampling, in the 0 to 15-, 15 to 27-, and 27 to 39-inch horizons of the frequently irrigated plots throughout the whole growing season (top), the frequently irrigated plots until the hard-dough stage (treatment No. 4), and the frequently irrigated plots until the silking stage (treatment No. 5). The rainfall (heavy lines) and the irrigations applied (broken lines) are shown below.

derived from data of table 15, there was a reduction in yields of 12.91, 9.28, and 6.35 hundredweights per acre in the plots where the irrigation was discontinued at the tasseling (treatment No. 6), silking (treatment No. 5), and hard-dough stages (treatment No. 4), respectively.

Table 15 also shows that nitrogen increased the corn yields in the irrigated plots.

DISCUSSION

Soil-moisture deficits from the tasseling to the hard-dough stages definitely had an influence on corn-yield reductions. There was a significant reduction

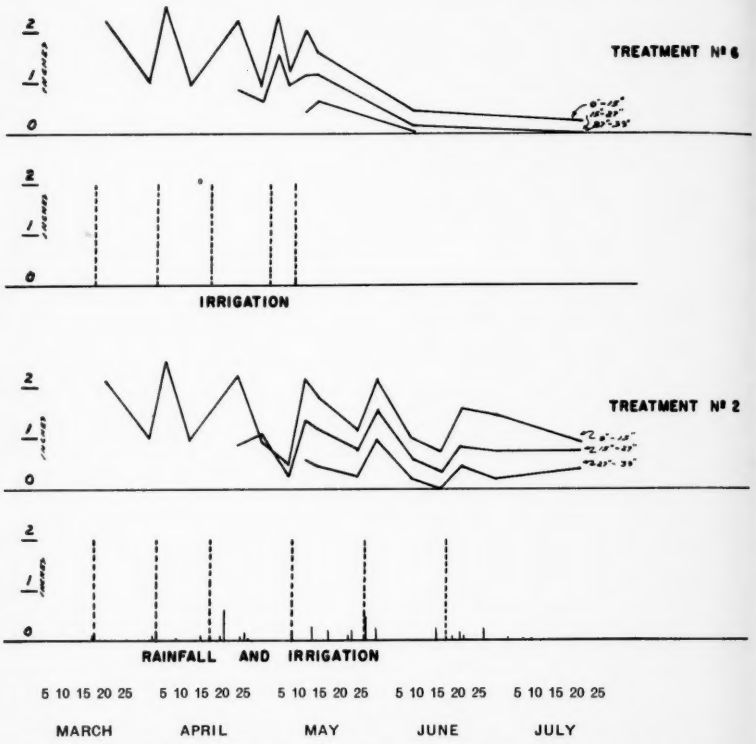
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FIG. 2.—Available water as indicated by soil sampling, in the 0 to 15-, 15 to 27-, and 27 to 39-inch horizons of the frequently irrigated plots until the tasseling stage (top) and the intermediately irrigated plots (treatment No. 2). The central chart presents the irrigations applied to the frequently irrigated plots until the tasseling stage (top figure). The bottom chart presents the rainfall (heavy lines) and the irrigations (broken lines) applied to the intermediately irrigated plots (treatment No. 2).

in yield of 22.5 and 16.2 percent in the plots where the irrigations were discontinued at the tasseling and silking stage, respectively. Robins and Domingo (*4*) found that soil-moisture deficits for periods of 1 or 2 days during the tasseling or pollination period resulted in as much as 22-percent yield reductions.

As in other corn experiments conducted during the same period (*6*), the

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maximum consumptive use occurred during the tasseling to hard-dough stages, decreasing in the last 45 days of the growing season. However, there was some difference in the total consumptive use in the other experiments reported and these also. This may have been caused by the different method used in soil sampling and the different set-up of the experimental plots used.

TABLE 15.—*Effect of irrigation at different growth stages and nitrogen fertilizer on the yield per acre (hundredweights) of 15-percent-moisture shelled corn grown in Santa Isabel clay at Lajas, P.R., 1957–58*

Irrigation treatment[1] No.	Effects of indicated pounds of nitrogen applied per acre, in experiment—					
	No. 1			No. 2		
	0	60 lb.	120 lb.	0	60 lb.	120 lb.
1	26.41	45.22	57.32	29.58	37.90	40.99
2	27.32	41.68	52.72	25.41	36.09	35.85
3	13.01	14.67	14.07	23.99	39.26	35.09
4	27.47	40.47	50.97	25.26	38.12	37.60
5	25.50	37.06	48.04	30.86	35.39	42.05
6	25.41	39.87	44.41	27.16	37.15	37.45
7	13.25	17.09	19.21	36.69	30.04	39.72
8	23.38	20.42	25.26	37.66	34.49	35.85
9	22.32	22.69	24.90	32.91	34.18	33.12

[1] The irrigation treatments were as follows: 1, Irrigated when 20 percent of the available moisture had been depleted from the active root zone. 2, Irrigated when 60 percent of the available moisture had been depleted from the active root zone. 3, Not irrigated after the crop was established. 4, Irrigated as in treatment No. 1 until hard-dough stage; no irrigation thereafter. 5, Irrigated as in treatment No. 1 until silking stage; no irrigation thereafter. 6, Irrigated as in treatment No. 1 until tasseling stage; no irrigation thereafter. 7, Not irrigated after the crop was established until hard-dough stage; as in treatment No. 1 thereafter. 8, Not irrigated after the crop was established until silking stage; as in treatment No. 1 thereafter. 9, Not irrigated after the crop was established until tasseling stage; as in treatment No. 1 thereafter.

Although, for lack of irrigation water, the irrigation levels were not maintained as originally planned, as shown in figures 1 and 2, more available moisture was maintained throughout the whole growing season in the frequently irrigated plots (treatment No. 1) than in the intermediately irrigated plots (treatment No. 2). However, the differences in corn yields were not statistically significant. There were significant yield reductions in the plots where the available moisture was maintained low from the tasseling to the hard-dough stages (May 23 to June 10). In the plots where the available moisture decreased after the hard-dough stage (June 10) the reductions in

FIG. 3.—Response of corn to irrigation at different growth stages when fertilized at a rate of 120 lb. of nitrogen per acre in experiment No. 1: 9, Not irrigated after the crop was established until the tasseling stage; 8, not irrigated after the crop was established until the silking stage; 7, not irrigated after the crop was established until the hard-dough stage, each one being irrigated after reaching the respective growth stage; 1, frequently irrigated throughout the whole growing season.

FIG. 4.—Response of corn to irrigation at different growth stages when fertilized at a rate of 120 lb. of nitrogen per acre in experiment No. 1: 6, Frequently irrigated from planting to the tasseling stage; 5, frequently irrigated from planting to the silking stage; 4, frequently irrigated from planting to the hard-dough stage, but unirrigated after reaching the respective growth stage; 1, frequently irrigated throughout the whole growing season.

yields were not significant. In other words, there was no significant difference in corn yields between the frequently irrigated plots (10 irrigations applied) and the plots frequently irrigated until the hard-dough stage (7 irrigations), with no more irrigations thereafter.

Figures 1 and 2 also show that the available moisture in the 27- to 39-inch horizon was maintained very low. This was probably due either to deficient irrigations applied, or to a different moisture-holding capacity, since no moisture-release data were determined for that horizon. Because the physical characteristics of the 27-to-39-inch horizon were very similar to the 15-to-27-inch horizon, the same soil-moisture characteristics were used to calculate the available moisture on both.

As in the other experiments reported (*6*) nitrogen increased yields, and there was an interaction between irrigation and nitrogen. Further work is still needed, especially at different moisture levels throughout the available-moisture range with other growth stages being included.

SUMMARY

Two field experiments using nine irrigation and three nitrogen levels were conducted at Lajas Substation in order to determine the effects of irrigation at different growth stages on the production of field corn. Some plots were frequently irrigated, irrigated when 20 percent of the available moisture had been depleted from the active root zone; and intermediately irrigated, irrigated when the depletion was 60 percent throughout the whole growing season. Other plots were frequently irrigated from planting to the tasseling, silking, and hard-dough stages, not being irrigated thereafter; while others still were not irrigated after the crop was established until the tasseling, silking, and hard-dough stages; these plots were frequently irrigated thereafter. Nonirrigated plots were used as checks. The nitrogen levels tested were 0, 60, and 120 pounds per acre.

The data indicated that:

1. There was a response to irrigation in the first experiment conducted; however, in the second experiment 23 inches of well-distributed rainfall overshadowed the irrigation effects.

2. There was no significant difference in production between the frequently and the intermediately irrigated plots.

3. The frequently irrigated plots throughout the whole growing season (10 irrigations applied) significantly outyielded all the plots under other irrigation treatments, except those irrigated frequently until the hard-dough stage (7 irrigations) with no irrigation thereafter, and the intermediately irrigated plots throughout the whole growing season (6 irrigations).

4. There were reductions in yields of 12.91 and 9.28 hundredweights per acre in the plots where the irrigations were discontinued at the tasseling and silking stages, respectively.

5. The maximum consumptive use of water occurred during the tasseling to hard-dough stages, decreasing in the last 45 days of the growing season.

6. There was a positive response to nitrogen applications, there being an interaction between irrigation and nitrogen.

RESUMEN

Dos experimentos de campo para estudiar el efecto de la aplicación de riego a distintas etapas de crecimiento en combinación con tres niveles de nitrógeno en la producción de maíz fueron llevados a cabo en la Subestación de Lajas. Se usaron los siguientes tratamientos de riego: Frecuente, cuando el 20 por ciento del agua asimilable había sido usada; intermedio, cuando el 60 por ciento del agua asimilable había sido usada; y testigo, parcelas sin riego. Además, se les aplicaron riego frecuente a otras parcelas hasta las siguientes etapas de crecimiento: Espigamiento, barba y hasta que el grano estuvo formado y duro (a los 80 días de sembrado), suspendiéndose el riego una vez llegó a su respectiva etapa de crecimiento. En otras parcelas se empezó la aplicación de riego frecuente una vez el maíz llegó a las etapas de crecimiento antes mencionadas, continuándose el riego hasta la cosecha. A estas parcelas, al igual que a las parcelas testigos, se les hizo una aplicación de riego inicial para establecer las plantas. Los niveles de nitrógeno incluídos fueron 0, 60 y 120 libras por acre. Los resultados obtenidos fueron los siguientes:

1. La aplicación de riego aumentó significativamente la producción de maíz en el primer experimento llevado a cabo; sin embargo, en el segundo experimento no hubo diferencia significativa en la producción debido a que cayeron 23 pulgadas de lluvia bien distribuídas durante el período de crecimiento.

2. No hubo diferencia significativa entre la producción de las parcelas regadas frecuentemente y las regadas con menos frecuencia.

3. Hubo una diferencia significativa entre la producción de las parcelas regadas frecuentemente durante todo el período de crecimiento (10 riegos aplicados) y la producción de las demás parcelas con otros tratamientos de riego, con la excepción de las parcelas regadas frecuentemente hasta que el grano estuvo formado y duro (a las cuales se les aplicaron 7 riegos) y las parcelas con riego intermedio (6 riegos).

4. Hubo una reducción en los rendimientos de 12.91 y 9.28 quintales por acre en las parcelas que se les suspendió el riego en el estado de espigamiento y barbas, respectivamente.

5. El consumo de agua máximo fue durante el período del espigamiento hasta que el grano estuvo formado y duro, disminuyendo en los últimos 45 días del período de crecimiento.

6. Hubo un aumento significativo en la producción de maíz con la aplicación de nitrógeno, exhibiendo una interacción entre riego y nitrógeno.

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Canning Characteristics of the Cobre Variety Sweetpotato

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INTRODUCTION

Two new varieties of sweetpotatoes have been developed by the Agricultural Experiment Station of the University of Puerto Rico, the Rico and the Cobre. Moscoso (*1*)[2] showed that both varieties yield better than the U.P.R.3 variety which has been used almost exclusively for canning. Sánchez, *et al.* (*2*) studied the canning characteristics of the Rico variety and compared its chemical composition with that of the U.P.R.3. The present paper deals with the evaluation of the canning characteristics of the Cobre variety. A comparison is made of its chemical properties and canning quality with these characteristics of the standard processing variety, the U.P.R.3.

EXPERIMENTAL PROCEDURES

The two varieties of sweetpotatoes used in this study, the U.P.R.3 and Cobre, were obtained from the Gurabo Substation. They were harvested 135 days after planting, graded for size, and processed immediately. Only roots ranging in size from 1½ to 2 inches in diameter were used.

The sweetpotatoes were processed on a pilot-plant scale on a line with a capacity for processing 300 pounds per hour, as described by Sánchez, *et al.* (*2*), with the exception that the roots were preheated to inactivate the enzyme system to prevent discoloration, as suggested by Scott, *et al.* (*3*). The roots were washed in a soaking tank followed by a rinse in a rotary washer and then preheated in hot water. The roots were peeled in 10-percent lye at 210° F. in a rotary lye-peeler. From the lye-peeler the roots were discharged into a rotary washer provided with high-pressure water sprays to remove lye and loosen the peel. Trimming immediately followed peeling and washing. The roots were kept under water before and after trimming to prevent discoloration.

The sweetpotatoes were packed in No. 2 plain tin cans with C-enameled ends, 14-ounce fill-in weight. Thirty-five-degree Brix syrup at 200° F. was

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[2] Italic numbers in parentheses refer to Literature Cited, pp. 111.

added, and the cans were exhausted in a steam exhaust-box for 15 minutes to a center-can temperature of 165° F. Immediately after closing, the cans were retorted for 45 minutes at 240° F. and cooled to 100° F. The cans were air-dried and stored at room temperature of 85° F.

ANALYTICAL METHODS

ANALYSIS OF THE UNPROCESSED ROOTS

The roots were selected at random and ground in a food-chopper. Individual samples were taken for moisture, starch, sugar, B carotene, and vitamin C determinations. This method of selection and preparation of samples has been shown to involve relatively small errors in sampling and in the chemical determinations (*4*).

The A.O.A.C. procedures were used (*5*) for moisture determinations. Duplicate samples of the ground pulp weighing about 10 gm. each were dried to constant weight in a vacuum oven at 70° C. under a vacuum of 100 mm. Loss in weight was calculated as percentage of moisture.

For determination of reducing and total sugars, 25-gm. samples of the ground pulp were transferred to a 250-ml. volumetric flask, 115 ml. of 54.3-percent alcohol were added, and the mixture was heated in a steam bath for 1 hour. After cooling, 95-percent alcohol was added to make to volume, and the mixture filtered. Two-hundred-milliliter aliquots were evaporated on a steam bath to 20 to 30 ml., transferred to 100-ml. volumetric flasks, saturated neutral lead acetate solution was added, and the mixture was allowed to stand 15 minutes. The solution was diluted to mark with water and filtered. The filtrate was deleaded with Na_2CO_3 and filtered. Reducing sugars were determined in the filtrate without inversion. Total sugars as invert were determined after inversion with hydrochloric acid. Sugars were determined by the Munson and Walker procedure (*6*).

B carotene was determined in samples of the ground pulp by the Moore and Ely procedure, but using acetone as the extractant (*7*, *8*).

Starch was determined by the method described by Carter and Neubert (*9*) for the determination of starch in apples. The standard spectrophotometric curve was prepared using a Spectronic 20 at 590 mμ.

For total-acidity determination a 10-gm. sample of the ground pulp was made into a slurry with distilled water and titrated with 0.1 N NaOH to pH 8.1 in a titrometer. Titrable acidity is expressed as percentage of anhydrous citric acid.

Color was measured in a Hunter Color and Color Difference Meter as follows: A whole root was cut lengthwise in halves, color was measured for the exposed flesh with the meter calibrated for R_d, a and b, with a white standard. Readings from several roots of the same variety were averaged.

Vitamin C was determined in samples of the ground pulp by the Ballentine iodate method (*10*).

ORGANOLEPTIC TESTS

The quality of the canned product was determined organoleptically by trained tasters from our own staff. Two methods were used to determine product quality and preference, the pair test and the hedonic scale. Samples of processed sweetpotatoes were presented to the tasters in an air-conditioned tasting booth under red light. The color of the samples were scored under daylight. In the pair tests, a sample of each variety was presented to the tasters and they were requested to taste both samples and to indicate their preference on the basis of tenderness, flavor, color, and appearance.

In the hedonic-scale method of measuring food preference, a 9-point scale expressed in terms of like and dislike was used to determine tasters preference for flavor and color of the processed sweetpotatoes. Samples were presented to the tasters under the conditions described above, and each sample was tasted and rated before the next was served.

RESULTS

COMPOSITION OF THE UNPROCESSED ROOTS

The results of the chemical analyses are given in table 1. These values represents the average of three analyses for each variety, harvested 135 days after planting. The Cobre variety was found to be higher in moisture, reducing sugars, and vitamin C than the U.P.R.3; it was lower in B carotene; it had a similar starch content.

Both varieties were found similar in total acidity and pH. When the varieties were compared for color, the U.P.R.3 was found to have a deeper orange color. The difference in the intensity of orange color was indicated by higher *a* and *b* Hunter values, indicating a higher concentration of the red and yellows. This was to be expected from the higher B carotene content of the U.P.R.3 variety.

PROCESSING CHARACTERISTICS

Both varieties were preheated before peeling to inactivate the cathecol oxidase. The Cobre variety had to be preheated 30 minutes at 135° F. for complete inactivation of the enzyme system while the U.P.R.3 had to be preheated for 30 minutes at 165° F. to obtain a similar level of inactivation. This suggests a slower rate of heat penetration in the U.P.R.3 variety, which may be due to its lower moisture content.

Both varieties were peeled in 10-percent lye at 210°F. for 6 minutes. Both varieties were found easy to peel. After lye-peeling it was easy to remove all rags and excess lye from the roots by washing with water sprays in a rotary washer. The peeled roots were always found to be of bright

orange color, free from discoloration. No difference in behavior during the peeling was observed, except that peeling losses were higher for the U.P.R.3, as seen from the data in table 2. Trimming losses were found higher also for the U.P.R.3 variety.

In these experiments a 56.66-percent recovery of roots for canning was obtained from the U.P.R.3 variety. Sánchez, *et al.* (*2*) reported 63.00-percent recovery of roots for canning of the U.P.R.3. This difference in yield may occur because the sweetpotatoes for the two experiments were planted

TABLE 1.—*Chemical composition of raw roots of Cobre and U.P.R.3 sweetpotato varieties expressed on wet basis*

Characteristic measured	Cobre	U.P.R.3
Moisture	73.50	68.53
Total acidity as anhydrous citric.....percent	.134	.1277
Reducing sugar..... do.	1.12	.67
Total sugar as invert..... do.	4.169	3.55
Starch..... do.	22.69	21.11
Vitamin C.....mg./100 gm.	34.89	24.15
B carotene.....mg./100 gm.	9.76	10.08
Specific gravity	1.029	1.041
Hunter color		
R_d	44.38	41.64
a	24.80	28.78
b	28.45	30.27

TABLE 2.—*Percentage peeling and trimming losses, and recovery of roots for canning, for raw roots of Cobre and U.P.R.3 sweetpotato varieties (average figures from 3 runs)*

Item	Cobre	U.P.R.3
Peeling loss	27.21	29.06
Trimming loss	13.55	14.28
Recovery of roots for canning	59.24	56.66

in different regions of the Island. The sweetpotatoes used by Sánchez, *et al.* (*2*) were planted at the Isabela Substation located in the northwest section of the Island, while the sweetpotatoes used in this experiment were planted at the Gurabo Substation, in the central eastern section of the Island, where the soil and the climatic conditions differ somewhat from those of the northwest.

SYRUPING, EXHAUSTING, AND COOKING

No difference could be observed between the two varieties during syruping, exhausting, and cooking. Neither of the two varieties was found to

discolor appreciably during processing. Exhausting for 14 minutes to a can-center temperature of 160 to 165° F. was found adequate to ensure a good vacuum in the can. All packs in the course of this work were processed using the time and temperature recommended by Sánchez, *et al.* (*2*).

QUALITY OF THE CANNED PRODUCT

As previously indicated, two methods were used to determine product quality and preference: The pair test and the hedonic scale. In using the pair test, a sample of each variety was presented to the tasters at the same time. They were required to indicate their preference for the different quality criteria.

The results of pair-test comparisons are given in table 3, in which the percentage of tasters selecting a sample is indicated. Statistical analyses of

TABLE 3.—*Results of pair-test comparisons on canned Cobre and U.P.R.3 sweetpotato varieties, showing percentage of tasters selecting samples for indicated quality criteria*

Quality criteria	Percentage of tasters selecting sample—					
	Cobre A	U.P.R.3 A′	Cobre B	U.P.R.3 B′	Cobre C	U.P.R.3 C′[1]
More attractive color	46.67	53.33	20	80	0	100*
Better flavor	40.00	60.00	70	30	18.19	81.81**
More tender	48.33	51.67	75	25	37.39	62.61

[1] *Significant difference between samples at 5-percent level and ** at 1-percent level.

the data show that no difference between tests A-A′ and B-B′. In test C-C′, the U.P.R.3 was found to be more attractive in color, the result being significant at the 1-percent probability level. It was also found that the U.P.R.3 had better flavor, with the difference being significant at the 5-percent probability level.

Although it was possible to establish certain preferences between the varieties in the pairs studied, no conclusive results were obtained to indicate that one variety was better than the other.

When the samples were submitted to organoleptic evaluation no significant difference in preference was obtained using the 9-point hedonic scale for color and flavor. Both varieties were rated alike and highly accepted.

The difference between varieties was so small that it could hardly be detected in a pair or a hedonic test. It is to be expected that consumers would be unable to detect any difference in quality between varieties.

The results of these tests indicate that both varieties are similar from the standpoint of processing characteristics and flavor. The Cobre variety

was found to be similar in many respects to the U.P.R.3 and, therefore, can be used successfully by canners.

SUMMARY

The chemical composition of unprocessed roots, as well as the canning quality of the Cobre variety of sweetpotatoes was studied. Its behavior during processing, as well as the quality of the canned product, was compared with that of the U.P.R.3 variety. Unprocessed roots of the Cobre variety were found higher in moisture, reducing sugars, and vitamin C, but lower in B carotene. Recovery of roots for canning was 2.58 percent higher for the Cobre than for the U.P.R.3 variety. There was very little difference in the quality of the syrup-packed potatoes of the two varieties. Tasters displayed no preference for either variety.

RESUMEN

Se estudió la composición química de la batata de la variedad Cobre, así como su comportamiento durante su elaboración y la calidad del producto enlatado. Se comparó su comportamiento durante el enlatado y calidad del producto enlatado con los de batatas de la variedad U.P.R.3. La batata de la variedad Cobre contiene en su estado natural más humedad y un porcentaje de azúcares reductoras e invertidas totales más alto que la variedad U.P.R.3. Sin embargo, esta última es más rica en B caroteno.

Se pudo establecer que el rendimiento de la batata elaborada fue un 2.58 por ciento mayor en la variedad Cobre. No se pudo establecer diferencia alguna en la calidad del producto envasado en sirop de estas dos variedades.

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Use of Allyl Alcohol as a Pre-emergent Weedkiller in Coffee Seedbeds[1]

J. E. Barbosa-Cupeles and M. A. Lugo-López[2]

INTRODUCTION

Weed control in coffee seedbeds is a very expensive farm item. Hand-picking of weeds is the conventional method used throughout the coffee-growing regions. Experiences in the control of weeds in tobacco seedbeds[3] led to the investigation reported in this paper. Allyl alcohol is a water-soluble contact weedkiller available in local markets either in its pure chemical form or in other formulations.

This paper reports data from an experiment conducted at the Gurabo Substation in an effort to determine whether allyl alcohol as a pre-emergent weedkiller is effective in coffee seedbeds, whether it adversely affects seed germination and seedling vigor, and which is the optimum concentration of the chemical that should be used.

MATERIALS AND METHODS

The experimental field was located on an alluvial soil at the Gurabo Substation farm in east-central Puerto Rico. The experimental design was a paired-plot one with four treatments, each replicated nine times. The treatments were included in 18 blocks. The plots were 10 feet by 10 feet in size (fig. 1). The treatments consisted of varying rates of application of the weedkiller as follows: 0, 1, 2, and 3 gallons of allyl alcohol per 1,000 square feet of area, respectively.

The allyl alcohol was diluted in 50 gallons of water and spread evenly over the plots on December 10, 1958. Seed of the Bourbon coffee variety were sown 6 days later. A 9-10-5 fertilizer was applied at the rate of 1,000 pounds to the acre. Germination was excellent and all plants survived. Weeds were hand-picked at two periods: January 15 and April 20, 1959 and weight of weeds per plot were recorded. For statistical analysis the weights of the weeds per plot were combined.

[1] Contribution from the Gurabo Substation.

[2] Research Assistant in Agronomy and Soil Scientist in Charge of the Gurabo Substation, respectively, Agricultural Experiment Station, University of Puerto Rico, Gurabo, P.R. Appreciation is expressed to Dr. H. R. Cibes, Associate Plant Physiologist, for his advice as to the concentrations of allyl alcohol to test, and to Dr. B. G. Capó, Associate Director, and Mr. A. Cruz Miret, Assistant Agronomist, for helping in the design of the experiment and the interpretation of the data.

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FIG. 1.—General view of the experimental field where the effectiveness of allyl alcohol, in different concentrations, was tried as a weedkiller in coffee seedbeds.

TABLE 1.—*Weight of weeds from control plots and from those receiving allyl alcohol at various concentrations*

Treatment identification No.	Rate of allyl alcohol applied	Adjusted mean weight of weeds[1]	Outyielded indicated treatment at the—	
			5-percent level[1]	1-percent level[1]
	Gal./1,000 sq.ft.	*Lb./1,000 sq.ft.*		
1	3	8.69	—	4
2	2	4.18	3	4
3	1	12.10	—	4
4	0	21.63	—	—

[1] Standard deviation of mean = 2.0108 lb; degrees of freedom for error = 15.

RESULTS AND DISCUSSION

Table 1 gives the combined adjusted mean weights of weeds for each treatment. A summary of the analysis of variance of the data obtained is given in table 2. The use of allyl alcohol at rates of 1, 2, and 3 gallons per 1,000 square feet of seedbed reduced weeds markedly. The mean differences between these treatments and the control plots were highly significant.

The use of 2 gallons of allyl alcohol was significantly better than the use of only 1 gallon per 1,000 square feet. There was no reduction in the germi-

TABLE 2.—*Summary of the analysis of variance of the experimental data herein reported*

Source	Degrees of freedom	Sum of squares	Mean square	*f*
Total	35	2,905.41		
Blocks (unadjusted)	17	1,554.52		
Treatments (adjusted)	3	986.98	328.9933	13.5608[1]
Error	15	363.91	24.2607	

[1] Indicates highly significant differences between treatments.

FIG. 2.—The use of allyl alcohol as a pre-emergent weedkiller on large scale at the Gurabo Seed Farm was very effective in keeping weeds under control, thus reducing expenses considerably.

nation of the coffee seed and no adverse after-effects were observed on the seedlings.

The reduction in weeds attributable to the use of allyl alcohol results in considerable savings, as weeding must be done by hand in coffee seedbeds, and it is quite an item of expense. Furthermore, more healthy and vigorous

seedlings can be obtained because allyl alcohol-treated seedbeds have so few weeds that there is but little competition for essential nutrients and moisture during the development and growth of the seedlings.

Following these tests allyl alcohol was used on a more extensive scale in coffee seedbeds established as part of the program of the Gurabo Seed Farm and the small-scale experimental results were fully confirmed (fig. 2). Weeding expenses were reduced as compared to previous years where no weedkillers were used in coffee seedbeds.

It might be well to emphasize here that allyl alcohol is poisonous and irritant, and is a lachrymator. Considerable care must be exercised in handling this chemical. Farmworkers should be properly trained and duly protected.

SUMMARY

Data are reported here from a field experiment in which allyl alcohol was used in coffee seedbeds as a pre-emergent weedkiller. Rates of 1, 2, and 3 gallons per 1,000 square feet of seedbed were used in comparison to check plots where no allyl alcohol was applied. The use of allyl alcohol reduced the weeds in all treated plots, the mean differences between them and the control plots being highly significant. Using 2 gallons of allyl alcohol was better than using 1 gallon per 1,000 square feet of surface.

RESUMEN

Se informan aquí datos de un experimento de campo donde se usó el alcohol alílico como yerbicida pre-emergente en semilleros de café. El uso del alcohol alílico, a razón de 1, 2 y 3 galones por cada 1,000 pies cuadrados de semillero, se comparó con las parcelas testigos que no recibieron yerbicida. El uso del alcohol alílico redujo los yerbajos en todas las parcelas tratadas, registrándose diferencias altamente significativas entre éstas y las parcelas testigos. El uso de 2 galones de alcohol alílico por cada 1,000 pies cuadrados de superficie fue significativamente superior al de un galón.

Use of the Root-Dipping Inoculation Method in Testing Tomatoes for Wilt Resistance

José Adsuar[1]

INTRODUCTION

The wilt disease caused by *Pseudomonas solanacearum* E.F.S. is at present the most important bacterial disease of tomatoes in Puerto Rico, especially in the humid sections of the Island.

The search for a variety of tomato resistant to bacterial wilt under our conditions has not been successful up to now in Puerto Rico (*1*, *2*, *3*, *4*, *5*)[2], although the potential value of a resistant variety was recognized in the earliest papers concerned with the control of bacterial wilt. It is to be noted that the work in North Carolina (*6*) on this problem was more hopeful.

Work is in progress at this Experiment Station to develop tomato (*Lycopersicon esculentum* Mill.) lines resistant to bacterial wilt. Greenhouse tests have been used for the screening of lines in the search for resistance. In this work use has been made of the inoculation techniques for determining resistance as described by Winstead and Kelman (*6*).

These techniques included inoculation with pure cultures by: 1, Forcing a sharp needle into the stem through a drop of bacterial suspension placed in the axil of the second or third expanded leaf below the stem apex; 2, cutting the lateral roots with a scalpel (root injury) along one side of the plant to a depth of approximately 4 cm., and pouring 10 ml. of the bacterial suspension over the severed roots; or 3, dipping the roots in a bacterial suspension.

On the basis of their studies Winstead and Kelman concluded that, although stem inoculation consistently produced higher wilt readings in resistant plants, the greatest differentiation between resistant and susceptible plants was obtained by root inoculation following the root-injury technique.

Preliminary inoculation trials[3] to determine the effectiveness of the root-injury technique were conducted using the highly susceptible Marglobe variety. For undetermined reasons poor results were consistently obtained.

PROCEDURE

Much better results, however, were secured when the roots were dipped in the bacterial suspension and the seedlings planted after treatment. The

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[2] Italic numbers in parentheses refer to Literature Cited, p. 118.

[3] Appreciation is expressed to Dr. J. Enrique Pérez and especially to Mrs. Amelia Cortés-Monllor of the Department of Plant Pathology and Botany, for their advice and invaluable aid in the preparation of the inoculum.

following technique was used: Seed of tomato variety Marglobe were planted in enameled basins 9¾ inches in diameter filled with steam-sterilized river sand. The seed germinated well and the young plants were kept in vigorous condition by drenching with one application of a proprietary nutritive solution (Nutrileaf), 1 tablespoonful in 1 gallon of water. When the plants were 4 weeks old they were gently pulled from the sand and dipped for 10 minutes in a suspension of *Pseudomonas solanacearum* E.F.S., isolate No. 65, a virulent isolate obtained locally from a bacterial wilt-diseased tomato plant. This isolate has been kept in the laboratory under oil for more than a year.

The inoculum was obtained from 4 to 5 day-old cultures grown on potato-dextrose agar in Blake bottles. The bacterial suspension was made following the method of Winstead and Kelman (*6*) by adding 100 ml. of sterile dis-

FIG. 1.—A, Control and B, inoculated tomato seedlings.

tilled water to each bottle and then diluting the suspension from each bottle by the addition of 300 ml. of water. In this case a slight deviation from the Kelman and Winstead technique was used in that sterile distilled water was employed instead of tapwater.

After roots of the plants had stayed in the suspension for 10 minutes they were planted immediately in the sand, 100 to 150 plants per basin, and placed in a screened house. The basins were covered with glass jars to maintain high humidity. Uninoculated plants which had been pulled out and replanted, and otherwise kept under the same condition were grown in basins beside the inoculated plants as controls.

RESULTS

The experiments were repeated six times at different times of the year and in each test more than 90 percent of the tomato seedlings consistently wilted and died (fig. 1) from 7 to 10 days after inoculation. The pronounced stunting and cessation of growth of uninoculated controls accompanying the root-dipping technique which, according to Winstead and Kelman, made

them discontinue its use, was negligible or not observed at all in our experiments.

In view of the above-mentioned experiences we believe that the use of the root-dipping inoculation method might be of value in testing for resistance to bacterial wilt of tomatoes under our conditions.

SUMMARY

This paper presents some experiments dealing with the effectiveness of the root-dipping method of inoculation in testing tomatoes for resistance to bacterial wilt. In the several trials performed more than 90 percent of the inoculated seedlings wilted and died when root-dipped in the suspension of the bacterial pathogen. We did not observe the pronounced stunting of growth of seedlings which, according to other investigators who have used this method, precludes the employment of this technique of inoculation. In our opinion the root-dipping method of inoculation should be a valuable adjunct in testing for resistance to bacterial wilt.

RESUMEN

Este estudio presenta los resultados de pruebas en las cuales se usó el método de inoculación por las raíces para determinar la resistencia del tomate al germen causante de la marchitez bacteriana. Los resultados obtenidos de los distintos ensayos demostraron que sucumbió más del 90 por ciento de las plántulas inoculadas con el patógeno por este método. No pudimos observar en ninguno de los ensayos el notable retardamiento en el desarrollo de las plántulas inoculadas y lo cual, de acuerdo con otros investigadores, descarta el uso de este método de inoculación. Creemos a la luz de nuestra experiencia que el método de inoculación por las raíces es un medio eficaz para determinar la resistencia del tomate a la marchitez bacteriana.

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The Biosynthesis of Steroidal Sapogenins by Homogenates of *Dioscorea* Tubers

Bruce Roark[1] *and M. P. Morris*[2]

INTRODUCTION

Several years ago other investigators reported that fermentation for 1 to 21 days of comminuted tubers of *Dioscorea* led to increased yields of diosgenin (*1, 2*)[3]. It can be reported now that fine comminution alone leads to these same apparent increases, and that fermentation for 1 to 21 days is not necessary to produce increased yields of diosgenin. Evidence has been obtained that a rapid enzymatic synthesis of steroidal compounds takes place in tubers which have been homogenized.

PROCEDURES

Representative subsamples of a single tuber of *D. floribunda* were analyzed (*3, 4*) after one of the following treatments: 1, Homogenized fresh with an equal quantity of water and hydrolyzed immediately in 2 N HCl; 2, homogenized fresh with an equal quantity of water and rapidly dried to constant weight at 85° C. before hydrolysis; 3, killed in autoclave at 15 pounds pressure for 5 minutes, then homogenized with an equal quantity of water and rapidly dried to constant weight at 85° C. before hydrolysis; 4, killed by homogenizing with an equal quantity of 2 N HCl, and hydrolyzed immediately; 5, sliced but not homogenized, rapidly dried to constant weight at 85° C., and ground to a fine dust before hydrolysis. The results are presented in the following tabulation showing yields of steroidal sapogenins of identical subsamples of a *Dioscorea* tuber (*D. floribunda*):

Treatment of fresh sample before analysis	*Steroids (percent on dry-weight basis)*[1]
1, Homogenized in water	6.0; 5.9; 5.8; 5.9
2, Homogenized in water and dried	6.2; 6.1; 6.2; 6.0
3, Autoclaved, homogenized, and dried	3.9; 3.7; 3.8; 3.8
4, Homogenized in 2 N HCl	2.9; 3.1; 3.2; 2.9
5, Sliced, dried, and ground	3.3; 3.6; 3.6; 3.4

[1] Determinations on fresh samples weighing 50 gm. each. Similar yields were obtained on fresh samples weighing 500 and 1,000 gm. The isolated product is a mixture of diosgenin and small amounts of at least three other steroids. Similar results were obtained with *D. composita*.

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[3] Italic numbers in parentheses refer to Literature Cited p. 120.

DISCUSSION

These results demonstrate that the highest yields of sapogenins are obtained when living tubers are homogenized as in methods 1 and 2. Significantly lower yields of sapogenins are obtained from *Dioscorea* tubers when the endogenous enzyme systems are destroyed by the addition of acid or by heating before the tissue is homogenized as in 3 and 4.

In a series of separate experiments homogenized tubers were fermented for periods up to 14 days. The yields of sapogenins were not significantly different from the yields of the controls which were assayed immediately after being homogenized.

Further evidence that sapogenins are formed in homogenized tuber tissue was obtained when fresh tubers were homogenized with 1-C^{14}-labeled acetate. The mixture was incubated overnight and a radioactive mixture of crude sapogenins was isolated from it. The sapogenins were still significantly radioactive after being crystallized several times from ether-petroleum ether mixtures. The steroids were then acetylated and the acetates were washed with water and recrystallized from petroleum ether. The acetates had approximately the same specific activity as the original steroids.

SUMMARY

It has been demonstrated that fermentation of comminuted tubers of *Dioscorea* for 1 to 21 days apparently increases the yields of diosgenin. We find that fine comminution produces the same result without fermentation.

This readily available enzyme system should be useful to workers in several fields. It provides a convenient tool for further studies on the biosynthesis of plant steroids. Furthermore, the incorporation of radioactivity into steroidal sapogenins should simplify the production of radioactive pharmaceutical products which are derived from them.

RESUMEN

Este valioso sistema enzimático puede tener muchas aplicaciones para trabajar en los más diversos campos científicos. Sería también, un sistema muy conveniente para futuros estudios sobre la biosíntesis de los esteroides en las plantas. Por otra parte, la incorporación de radiactividad dentro de esteroides sapogénicos podría simplificar la producción de aquellos productos farmacéuticos radiactivos derivados de éstos.

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The Effect of Fermentation on the Diosgenin Content of *Dioscorea* Tubers

B. Roark,[1] *H. Cruzado,*[2] *H. Delpin,*[2] *and M. P. Morris*[3]

INTRODUCTION

Diosgenin is the material employed for the preparation of several widely used pharmaceutical products. It can be isolated readily from several species of *Dioscorea* tubers. This steroid is the basis of an industry that each year fabricates products valued at more than $50,000,000. The wild, high-yielding tubers of commerce are native to Mexico and Central America, and have not yet been domesticated. Other high-yielding species of wild tubers are native to Africa. The domestication program here in Puerto Rico has been in progress for several years. The outlook is very promising.

PROCEDURES AND RESULTS

All of the commercial producers of diosgenin use essentially the same process for its isolation from wild *Dioscorea* tubers. This consists of three operations: 1, Maceration and fermentation of the tubers; 2, acid hydrolysis of the fermented mixture; 3, extraction of the diosgenin with petroleum ether.

The fermentation step has been an important part of the standard isolation procedure for almost 20 years. Its use is based on the belief that fermentation increases the amount of diosgenin that can be isolated from the tubers. This paper describes a study of the fermentation procedure, and presents data which illustrate for the first time the true effects of fermentation on diosgenin content.

Fresh tubers, 2 years old and weighing 30 pounds, were sliced, divided into three equal portions, and then homogenized with an equal weight of water in a high-speed blender for 5 minutes. The macerated tubers were then placed in 10-gallon porcelain pots. The total weight, the percentage moisture, and the total diosgenin were recorded for each pot. The pots were then stored for 7 days at 30° C. in a dark room. At the end of 7 days the contents of one pot were weighed and analyzed[4]. After fermenting for 2 weeks the contents of the second pot were analyzed, and the contents of the third jar

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[4] Rothrock, J. W., Hammes, P. A., and McAleer, W. J., *Ind. Eng. Chem.*, **49**, 186–88, 1957.

were analyzed after fermenting for 3 weeks. This experiment was repeated three times with both *Dioscorea composita* and *D. floribunda*. The results of all experiments were for all practical purposes the same. Typical data are presented in table 1.

TABLE 1.—*Fresh weight, dry weight, and diosgenin contents (grams) of 2 species of* **Dioscorea** *after various treatments*

Treatment of fresh tuber before analysis	*D. composita*			*D. floribunda*		
	Fresh weight	Dry weight	Total diosgenin	Fresh weight	Dry weight	Total diosgenin
1, Sliced, dried, and ground	600	197.6	5.2	600	200.0	5.5
2, Homogenized, dried, and ground	600	200.0	8.4	600	204.0	8.9
3, Homogenized and fermented 7 days	600	183.6	8.2	600	198.3	8.9
4, Homogenized and fermented 14 days	600	182.5	8.3	600	174.7	8.5
5, Homogenized and fermented 21 days	600	180.4	7.6	600	173.4	8.0
6, Homogenized and fermented 28 days	600	175.5	7.0	600	149.3	7.6

DISCUSSION

The data show that the total weight of diosgenin in the fresh tubers is not changed by fermentation periods extending up to 21 days. It will be noted that the percentage of diosgenin apparently increases during fermentation. It is obvious, however, that this increase does not reflect a change in the quantity of diosgenin present, but rather indicates a decrease in the dry weight of total solids. The data show that fermentation does not increase the weight of diosgenin present in *Dioscorea* tubers.

SUMMARY

The total weight of diosgenin in fresh *Dioscorea* tubers remains unchanged during fermentation periods of up to 21 days, but the percentage of diosgenin seemingly increases. This apparent increase is attributed to a decrease in the dry weight of total solids present only.

RESUMEN

Los datos experimentales muestran que el peso total de diosgenina en los tubérculos frescos de *Dioscorea* no cambia durante el período de fermentación. Se nota que el porcentaje de diosgenina aumenta durante la fermentación. Por otra parte, este aumento no depende de un cambio de la cantidad de diosgenina presente, sino del peso seco de los sólidos totales, el cual disminuye. Por consiguiente, los datos experimentales muestran que la fermentación no aumenta el peso de diosgenina presente en los tubérculos de *Dioscorea*.

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(*Continued from inside front cover*)

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